Investigation of lipodystrophy syndrome in a multicentre prospective cohort of HIV-infected children and adolescents living in Europe

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Declaration

I, Syed Naufil Mubashir Alam, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

This is an investigation into lipodystrophy syndrome (LS), defined as specific body fat alterations (BFA) and/or metabolic abnormality (MA), in HIV-infected children. Antiretroviral therapy (ART) has resulted in improved disease-free survival in HIV-infected individuals, but is an established risk factor for LS. The metabolic profile seen in LS patients is similar to that seen in the early stages of cardiovascular disease. As ART coverage improves, and because treatment is life-long, the importance of LS will become more relevant, especially as treated children survive into adulthood, and accumulate longer durations of exposure to multiple drugs.

Data from a prospective European multi-centre cohort of 426 HIV-infected subjects aged 2-18, has been analysed. The manifestation of LS using clearly-defined phenotypes is described, with estimates of prevalence and incidence. Risk factors for LS were identified in logistic, and proportional hazards regression models. The temporal relationship between BFA and MA was explored, and the impact of LS on serum lipids modelled using a multi-level approach. Finally interventions used to manage symptoms of LS were described.

The prevalence of LS at recruitment (median age 12.2 years) was 56.5% (95% CI: 51.7, 61.3): half had BFA alone, one-quarter had MA alone, and one-quarter had both. Over follow-up (median: 4.2 years) the incidence of BFA was 8.0 per 100 person-years (95% CI: 6.0, 10.7) and that of MA was 4.1 per 100 person-years (95% CI: 2.8, 5.9). Significant, independent risk factors associated with increased risk of LS outcomes included protease inhibitor (PI) or non-nucleoside reverse transcriptase inhibitor (NNRTI) use, age, clinical status, and White ethnicity. Decreased risk was associated with immunosuppression and detectable viral load. Several factors were associated with increased pro-atherosclerotic lipid concentrations over follow-up: however, NNRTI use and female sex were significantly associated with increased anti-atherosclerotic HDL-cholesterol. This thesis used data from a unique large prospective cohort to underline the increasing significance of LS in HIV-infected children and adolescents.

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95% CI	**95% C**onfidence **I**nterval
AHA	**A**merican **H**eart **A**ssociation
AHR	**A**djusted **H**azard **R**atio
AIDS	**A**cquired Immune **D**eficiency **S**yndrome
AOR	**A**djusted **O**dds **R**atio
ART	**A**nti**r**etroviral **T**herapy
ART-CC	**ART C**ohort **C**ollaboration
BFA	**B**ody **F**at Alterations
BMI	**B**ody **M**ass **I**ndex
cART	**C**ombination Anti**r**etroviral **T**herapy
CD4	**CD4** T-helper lymphocyte
CDC	**C**entres for **D**isease **C**ontrol
CHER	**Ch**ildren with **E**arly Anti**r**etroviral Therapy trial
CHIPS	**C**ollaborative **HI**V **P**aediatric **S**tudy
CHORUS	**C**ollaboration in **H**IV **O**utcomes **US** (cohort)
CIMT	**C**arotid **I**ntima **M**edia **T**hickness
CISAI	**C**oordinators (**I**talian) for the **S**tudy of **A**llergies and HIV **I**nfection
CRA	**C**hemokine Co-**r**eceptor **A**ntagonist
CRABP-1	**C**ytoplasmic **R**etinoic **B**inding **P**rotein **1**
CRP	**C**-**Rea**ctive **P**rotein
CT	**C**omputerized **T**omography
CVD	**C**ardio**v**ascular **D**isease
DAD	**D**ata Collection on **A**dverse Events of Anti-HIV **D**rugs (study)
DEXA	**D**ual **E**nergy **X**-ray **A**bsorptiometry
DNA	**D**eoxy**r**ibonucleic **A**cid
ECS	**E**uropean **C**ollaborative **S**tudy
EPPICC	**E**uropean **P**regnancy and **P**aediatric H**I**V **C**ohort **C**ollaboration
FI	**F**usion **I**nhibitor
FRAM	The Study of **F**at **R**edistribution **a**nd **M**etabolic Change in HIV infection
GLUT-4	**Glu**cose **T**ransporter Type **4**
HAART	**H**ighly **A**ctive **A**nti**r**etroviral **T**herapy
HDL	**H**igh **D**ensity **L**ipoprotein (cholesterol)
HIV	**H**uman **I**mmuno-deficiency **V**irus
HR	**H**azard **R**atio
HOPS	**H**IV-**O**ut**p**atient **S**tudy
HPPMCS	**H**IV **P**ediatric **P**rognostic **M**arkers **C**ollaborative **S**tudy
II	**I**ntegrase **I**nhibitor

IQR	**I**nter **Q**uartile **R**ange
KIDS-ART-LINC	**Kids**' **ART** in **L**ower **In**come **C**ountries (study)
LDL	**L**ow **D**ensity **L**ipoprotein (cholesterol)
IL-1	**I**nter**l**eukin **1**
IL-2	**I**nter**l**eukin **2**
IL-6	**I**nter**l**eukin **6**
LipoICoNa	**Lipo**dystrophy study in the Italian **Co**hort of ART-**na**ïve Patients
LS	**L**ipodystrophy **S**yndrome
MA	**M**etabolic **A**bnormality
MACS	**M**ulticentre **A**IDS **C**ohort **S**tudy
MDG	**M**illennium **D**evelopment **G**oal
MI	**M**yocardial **I**nfarction
MTCT	**M**other **T**o **C**hild **T**ransmission (of HIV)
mtDNA	**Mit**ochondrial **D**eoxyribo**n**ucleic **A**cid
NHANTES	**N**ational **H**ealth **a**nd **N**utri**t**ion **E**xamination **S**urvey
NIH	**N**ational **I**nstitutes of **H**ealth
NRTI	**N**ucleoside **R**everse **T**ranscriptase **I**nhibitor
NNRTI	**N**on-**n**ucleoside **R**everse **T**ranscriptase **I**nhibitor
NSHPC	**N**ational **S**tudy of **H**IV in **P**regnancy and **C**hildhood
OR	**O**dds **R**atio
PACTG	**P**ediatric **A**IDS **C**linical **T**rials **G**roup
PACTS	**P**erinatal **A**IDS **C**ollaborative **T**ransmission **S**tudy
PLATO II	**P**ursuing **La**ter **T**reatment **O**ptions **II**
PENTA	**P**aediatric **E**uropean **N**etwork for the **T**reatment of **A**IDS
PCP	*Pneumocystis jiroveci* pneumonia
PI	**P**rotease **I**nhibitor
PSD	**P**ediatric **S**pectrum of HIV **D**isease (study)
QQ	**Q**uantile-**q**uantile (plot)
RNA	**R**ibo**n**ucleic **A**cid
SREBP	**S**terol **Re**gulatory **P**rotein
TAPHOD	**T**REAT **A**sia **P**ediatric **H**IV **O**bservation **D**atabase
TNF	**T**umour **N**ecrosis **F**actor
UN	**U**nited **N**ations Organization
UNAIDS	Joint **U**nited **N**ations Programme on HIV/**AIDS**
UNICEF	**U**nited **N**ations **C**hildr**e**n's **F**und
VLDL	**V**ery **L**ow **D**ensity **L**ipoprotein (cholesterol)
WHO	**W**orld **H**ealth **O**rganization

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1. Human immunodeficiency virus and lipodystrophy syndrome

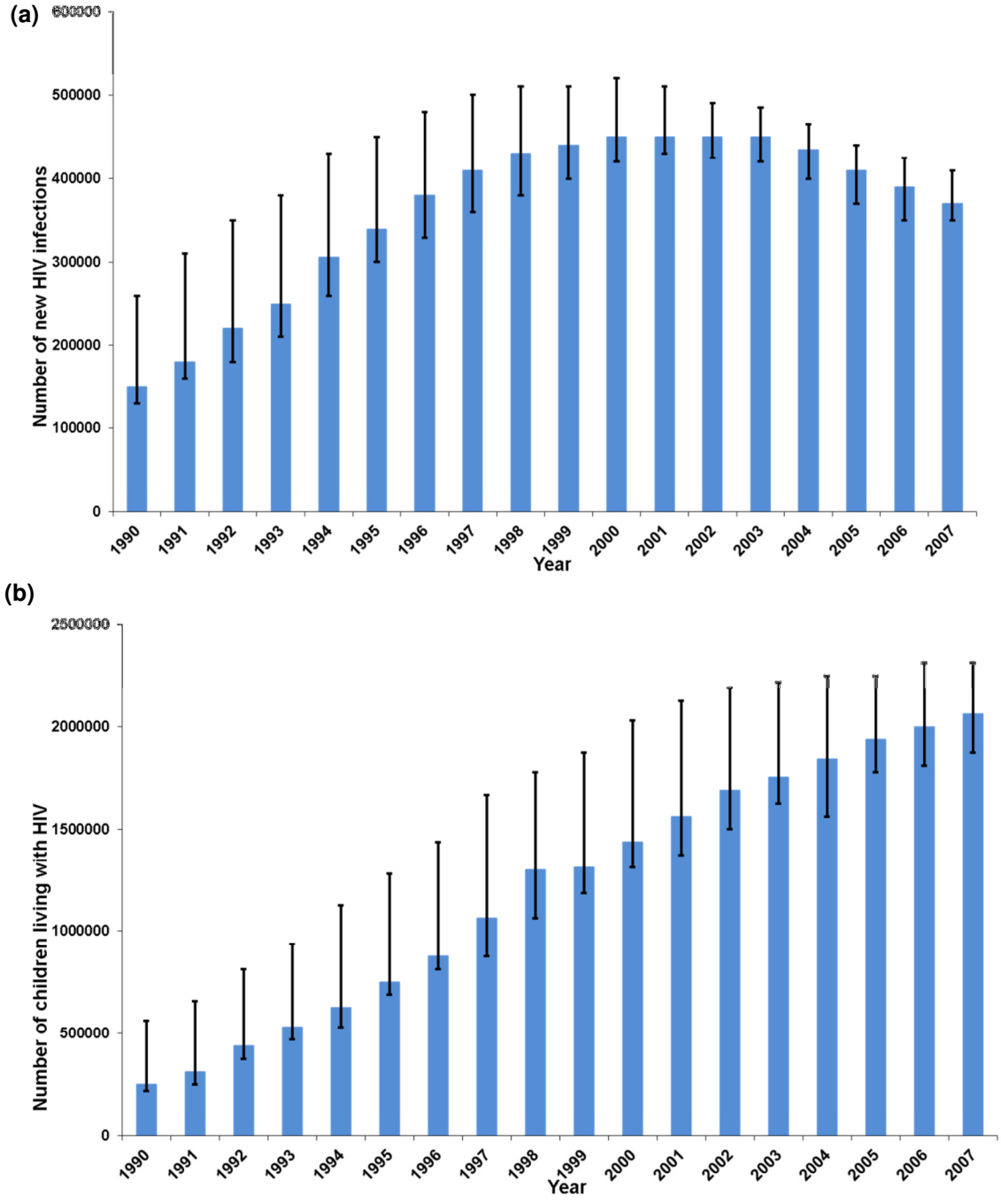
1.1 Epidemiology of HIV

The number of people estimated to be living with HIV in 2010 was 34 million (95% CI: 31.6, 35.2), of whom 3.4 million (95% CI: 3.0, 3.8) were children aged less than 15 years[1]. This marked an increase in prevalence from the previous year (total prevalence: 33.3 million 95% CI: 31.4-35.3 million, and paediatric prevalence: 2.5 million, 95% CI: 1.6-3.4[2]) reflecting improved prognosis following treatment (as opposed to an increase in incidence)[3]. The global incidence of HIV in children (i.e. aged <15 years) peaked in 2002-2002 (Figure 1-1) due to the stabilization of HIV prevalence amongst women and the prevention of vertical transmission[a4] (mother-to-child-transmission: MTCT), with paediatric prevalence increasing steadily over the past twenty years (Figure 1-1)[5].

The highest prevalence of HIV infection in children aged less than 15 years in 2010 was seen in Africa, with an estimate more than 150 times greater than the prevalence estimated in Europe (Table 1-1).

[a] An estimated 90% of newly infected cases of paediatric HIV in Sub-Saharan Africa per annum occur as a result of vertical transmission during or after pregnancy

Figure 1-1 Global (a) incidence and (b) prevalence of HIV infections among children aged <15 years between 1990 and 2007[5]



Estimates provided by the Joint United Nations Programme on HIV/AIDS (UNAIDS): bars indicate lowest and highest estimates from model.

Table 1-1: Estimated prevalence and incidence of HIV in children aged less than 15 years in 2010

	Prevalence		Incidence	
	Estimate	**(95% CI)**	**Estimate**	**(95% CI)**
Africa	3100000	(2800000, 3500000)	350000	(300000, 410000)
South East Asia	140000	(92000, 190000)	17000	(11000, 25000)
Americas	58000	(44000, 74000)	5000	(3200, 6900)
Eastern Mediterranean	42000	(28000, 57000)	7400	(5200, 9800)
Western Pacific	39000	(33000, 46000)	5000	(3800, 6200)
Europe	19000	(15000, 25000)	2400	(1900, 2900)

Data provided by the World Health Organization (WHO)/United Nations Children's Fund (UNICEF)/the Joint United Nations Programme on HIV/AIDS (UNAIDS)[1]

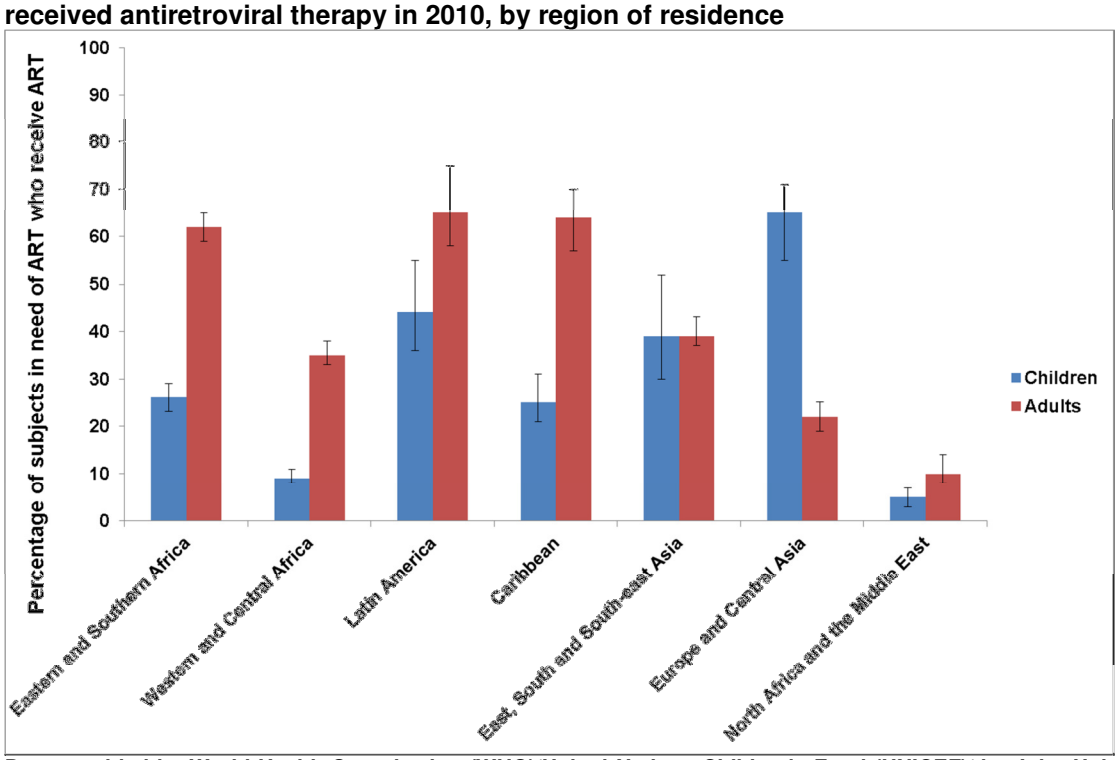
In 2010 the greatest incidence occurred in Africa followed by Southeast Asia, whilst the smallest incidence was seen in Europe[1]. Despite prevalence and incidence amongst children aged <15 years being greatest in Africa, access to antiretroviral therapy (ART) was consistently lower here compared to other World Health Organization (WHO) regions (Figure 1-2).The Joint United Nations Programme on HIV/AIDS (UNAIDS) estimated HIV prevalence was calculated using the *Estimates and Projection Package*, and also the *Spectrum Projection Package*. While the ability to investigate underlying assumptions and explore key indicators and trends are strengths of these models, the resultant projections must be treated with caution: some assumptions used in the calculation may be based on a small number of studies, patterns of prevalence by age and sex being assumed to be constant over time, and limited data on patterns of HIV infection in key high-risk groups e.g. age distribution among injecting drug users[6].

In 2001, member states of the United Nations Organization (UN) committed themselves to taking "extraordinary action to move towards universal access to HIV prevention, treatment and support" by 2010 as part of the *Political declaration on HIV/AIDS* [7]. Percentage coverage of ART increased in both children and adults between 2005 and 2008[8], through initiatives prompted by the sixth Millennium Development Goal (MDG) including the aim of achieving, by 2010*, the universal access to treatment for HIV/AIDS for all those who need it*[9,10]. UNAIDS estimate that 14.6 million (range; 13.2, 13.5) people were in need of ART in 2009, up from 14.3 million (range; 13.2, 15.4 million) in 2008, in low and middle income countries (using WHO 2010 guidelines regarding treatment[11,12b]). Only 5.2 million of these people received ART in 2009

[b] Eligibility for ART defined as either CD4 count ≤ 350 cells/mm3 irrespective of clinical symptoms, or WHO clinical stage 3 (moderate unexplained malnutrition/unexplained persistent diarrhoea/unexplained persistent fever/oral candidiasis/oral hairy leukoplakia/acute necrotizing ulcerative ginigivitis or periodontitis/pulmonary tuberculosis/ bacterial pneumonia/HIV-associated lung disease/lymphoid interstitial pneumonitis/ unexplained anaemia (<80d/L) and/or neutropenia (<1000/µL)/thrombocytopmenia (<50 000/µL)) or stage 4 (unexplained severe wasting or

compared to 4.0 million in 2008, although this represented an increase in the percentage coverage of ART from 28% (range; 26, 31) to 36% (range; 33, 39) between these two years. In 2009, 1.3 million (range; 0.8, 1.7) children aged less than 15 years were estimated to need ART, of whom 356 400 received therapy resulting in 28% (range; 21, 43) coverage[13,14].

Figure 1-2: Estimated proportion of HIV-infected adults and children (age <15 years) who received antiretroviral therapy in 2010, by region of residence



Data provided by World Health Organization (WHO)/United Nations Children's Fund (UNICEF)/the Joint United Nations Programme on HIV/AIDS (UNAIDS)[1]. 2008 WHO recommendation for ART in HIV-infected adults and adolescents in resource-limited settings: confirmation of infection, and either clinical signs of advanced disease or laboratory evidence of severe immunosuppression[11]. 2010 WHO recommendations for ART in HIV-infected adults and adolescents in resource-limited settings: CD4 count <350 cells/mm^3 regardless of presence or absence of clinical symptoms[12]

Most regions ART access is lower in children compared with adults. Europe is the only region where the converse was true in 2010[1], despite this being the region with the lowest estimates of HIV infection in children: this highlights the disparities between low-income with middle and high-income countries. Sub Saharan Africa is estimated to account for 89% of paediatric need for ART. Furthermore, rates of coverage in Latin America and the Caribbean, East, South/ South-East Asia, and, Europe and Central Asia are lower in children than in adults[13].

However, such comparisons over time are difficult to validate. Estimates of coverage in regions where numbers are low (such as Europe) are inaccurate, and furthermore, as recommendations for treatment have changed, comparisons over time may be inappropriate. Indeed,

malnutrition/pneumocystis pneumonia/recurrent bacterial infections/chronic herpes simplex/extrapulmonary tuberculosis/Kaposi's sarcoma/oesophageal candidiasis/central nervous system toxoplasmosis/HIV encephalopathy/cytomegalovirus infection/extrapulmonary cryptococcosis/disseminated endemic mycosis/cryptosporidiosis/isosporiasis/disseminated non-tuberculous mycobacteria infection/Candida infection/visceral herpes simplex/rectal fistula/cerebral or B cell non-Hodgkin lymphoma/progressive multifocal leukoencephalopathy/cardiomyopathy or nephropathy) disease irrespective of CD4 count

recommendations regarding eligibility criteria to commence ART have become broader over time. While this may be expected to increase uptake, in practice as eligibility criteria broaden, the number of entitled people who do not have access to treatment increase in resource poor settings. Thus, considering this caveat in tandem with advances in ART coverage, the goal of universal access remains elusive, particularly regarding levels of access among infected children.

1.2 HIV and AIDS

The disease which would later be known as Acquired Immune Deficiency Syndrome (AIDS) was first reported in 1981 as a set of case reports of *Pneumocystis jiroveci* pneumonia (PCP) (formerly known as *Pneumocystis carinii*) in gay men occurring in Los Angeles[15]. In addition to this rare form of fungal pneumonia, other opportunistic infections and cancers associated with AIDS were identified (e.g. mycobacterium infections, toxoplasmosis, invasive fungal infections, Kaposi's sarcoma and non-Hodgkin's lymphoma etc.).

Concurrently with these reports from Las Angeles in the early 1980s, reports of immunodeficiency in haemophiliac patients which was similar to the immunodeficiency seen in AIDS patients contributed to the theory of a blood-borne infection as the causative agent[16,17]. The occurrence of AIDS in heterosexual Africans who were not injecting drug users added to the growing evidence that there was also a heterosexual mode of transmission[18,19]. In 1983, a distinct retrovirus which had been seen in patients prior to the development of AIDS was first isolated[20]. Although a number of theories regarding the cause of AIDS were postulated[21], by 1984 evidence was growing linking infection by the retrovirus HIV[c] with the development of AIDS[22-24]. By 1986 the Centres for Disease Control (CDC) had developed a working definition for AIDS[25], which was revised in 1993[26]. Two strains of HIV were eventually isolated; HIV-1 and HIV-2. HIV-1 is more virulent, responsible for the majority of global HIV infections, while HIV-2 is mainly restricted to West Africa and may result in less aggressive disease[27].

[c] Previously known as Lymphadenopathy-Associated Retrovirus (LAV) in isolates taken from patients in France, and Human-T-cell Leukaemia Virus (HTLV-III) in isolates taken from patients in the USA.

1.3 Infection with HIV

HIV is a lentivirus composed of a protein capsid containing a lipid membrane enclosing two copies of single stranded ribonucleic acid (RNA) and the enzymes reverse transcriptase and integrase. The virus preferentially binds to host T-helper lymphocytes[28] (CD4 cells). CD4 cells have an essential function in the immune system, having roles in determining B-cell antibody class switching, activation of cytotoxic T-cells and activity of phagocytes. It is the HIV-mediated depletion of CD4 cells that leads to compromise of the host immune system and vulnerability to opportunistic infection[27,29-31]. The clinical endpoint of HIV infection is AIDS: while HIV infection may initially be symptomless, the transition to AIDS may (but not always) initially manifest as opportunistic infections, and cancers associated with the immune system[20].

1.3.1 Natural history of HIV infection

Following primary infection with HIV, there is a period of rapid replication of the virus[32-34] which is accompanied by a sharp decrease in CD4 lymphocyte cells[35-37]. This stage may be symptomatic, as seroconversion occurs, and last several weeks. The initial period of infection is known as the acute phase and culminates in wide dissemination of the virus and its seeding in lymphoid organs[38,39]. This is followed by a period of clinical latency where HIV level (viral load) remains low, and CD4 cell numbers recover, although HIV continues to be expressed by infected CD4 cells[40]. The asymptomatic latent period may last several years as viral load and CD4 cells levels stabilize. However, the value of the plateau in HIV-infected children is higher than in adults, and is accompanied by a slow sustained increase in CD4 cells[41]. Following a gradual rise in viral load and decrease in CD4 cells, constitutional symptoms, opportunistic disease, AIDS-related clinical illness, and ultimately death occur[42-46]. Viral load is closely associated with prognosis[47,48], while counts of CD4 cells are an indicator of the degree of immunosuppression: as viral load increases, there is a resultant reduction in CD4 cells which may culminate in a poorer prognosis. The estimated median interval between seroconversion and the onset of AIDS-related clinical disease in treatment–naïve adult patients is 11 years[49,50].

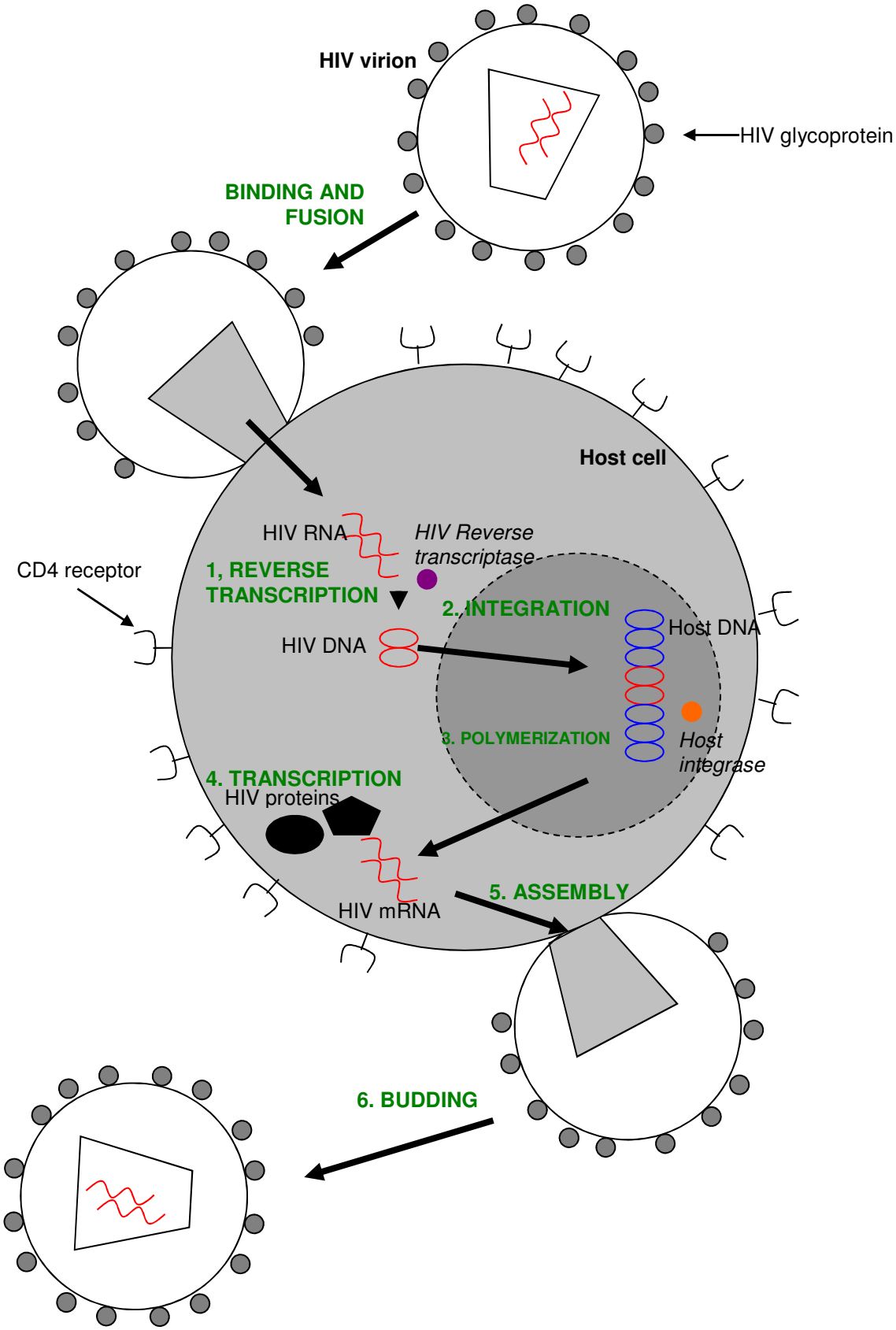
Around 20% of vertically-infected children progress to AIDS within the first year of life in the absence of treatment[51], a substantially higher proportion than seen in adults[52]. However, analysis of 990 children enrolled in the European Collaborative Study (ECS) suggested that the rate of progression in those children who do not rapidly progress may be slower than in adults, with less than 10% of HIV-infected children showing HIV-related symptoms before AIDS onset and after the first year of life, and less than 50% being symptomatic after AIDS diagnosis[53]. Furthermore, the HIV Paediatric Prognostic Markers Collaborative Study (HPPMCS) conducted a meta-analysis of longitudinal data collected for 3941 children which indicated that there were non-linear associations between risk of disease and both CD4% (the percentages of total lymphocytes that are expressing CD4 antigens) and viral load, with older children having lower

short-term risk of clinical progression than younger children at a given CD4%[54]. Further analysis of HPPMCS and comparison with data collected from adults suggested that, following adjustment for CD4 count, the effect of age on disease progression while not significant in children aged five year or older, was significantly associated with increases in AIDS-related disease or death in adults[55]. Indeed, these findings and the finding from a clinical trial reporting decreased hazard of AIDS-related outcomes in infants treated immediately with ART compared to infants with deferred treatment[56], has been important in updating paediatric treatment guidelines. Stage of maternal infection, maternal survival, background mortality rates and timing of infection (for example whether infection occurred *in utero,* intrapartum, or postnatal) have been identified as important predictors of paediatric survival times, especially in resource-poor settings[57,58].

1.3.2 Cellular mechanism of infection with HIV

Figure 1-3 illustrates the process of infection of a CD4 cell by HIV and highlights stages which are targets for therapeutic intervention.

Figure 1-3: Mechanism of infection of CD4 expressing cells by HIV (adapted from Levy 1993[42])



Key processes are shown in green: fusion, reverse transcription and integration are important targets for therapeutic action

HIV binds to the CD4 epitope of the host T-lymphocyte[28] initiating confirmation changes in the viral envelope and allowing for fusion between the virus and the cell. The HIV capsid enters the host cell cytoplasm where the viral reverse transcriptase enzyme transcribes the viral RNA to double stranded deoxyribonucleic acid (DNA). The newly produced HIV DNA then enters the CD4 cell nucleus where it becomes integrated into the host DNA by the viral integrase enzyme. Transcription of the host/viral DNA is catalysed by the host cellular RNA polymerase generating new HIV RNA. The host cellular apparatus are then used to construct and assemble new viral capsids which are released from the initial infected CD4 cell and can go on to infect further host CD4 cells[30].

1.3.3 Antiretroviral therapy

The aim of ART is to prevent increases in viral load by targeting specific steps in either the replication of HIV virions, or the infection of new cells, e.g. assembly of new virion particles, transcription of viral RNA, integration of nascent viral DNA into host cell DNA, or entry of virions into uninfected cells (Figure 1-3). The first antiretroviral drug, zidovudine, was found to be effective against HIV-1 in 1984, and licensed as an anti-HIV therapy in 1987[59].

Increasing coverage[60] of ART has resulted in considerably improved survival, better health and reduced need for hospitalization in both adults[61-63] and children[64-66]. Several studies have reported mortality to be significantly reduced in adults who are treated with ART[67-69]. An estimated 4-6 year survival benefit associated with specific ART regimens was calculated in a model using data from US life tables and from the Collaboration in HIV Outcomes US (CHORUS) cohort of 4791 individuals[70]. Indeed, a second mathematical model using CDC and other published surveillance data estimated that at least 3 million years of life have been saved in the United States as a result of treatment[71]. Analyses from the ART Cohort Collaboration (ART-CC), a collaboration of 14 cohorts of HIV-infected subjects in Europe and North America, have estimated that a HIV-infected individual living in a high-income country who is on ART and aged twenty years, may expect a lifespan two-thirds smaller than the average in their country of residence i.e. a life expectancy of 44.7 years[72]. Furthermore, a UK model predicted that median age of death for a man who becomes HIV-infected in 2010 via homosexual sex at age at 30 years, to be 75 years with an average of 7 years of life lost compared to an uninfected man[73]. As survival has improved and the HIV-infected population ages, the importance of co-morbidities including cardiovascular disease (CVD), diabetes, non-AIDS-defining malignancy, viral hepatitis and osteoporosis has been increasingly recognised [74].

Increased survival rates associated with ART in children have been reported in both the developing and developed world. The 5-year survival rate, from the initiation of ART, in 1752 children enrolled in the multi-country TREAT Asia Pediatric HIV Observation Database (TAPHOD) project was 91.7% (95% CI: 90.0, 93.2), with mortality being highest in the first three months after start of treatment[75]. Similarly, the 2-year survival rate was 93.1% (95% CI: 94.1,

98.9) in 1058 children enrolled across 8 cohorts participating in the Kids' Antiretroviral Treatment in Lower-Income Countries (KIDS-ART-LINC) Collaboration across Sub-Saharan Africa[76]. Furthermore, rates of survival of HIV-infected children improved in the 1997-2001 birth cohort relative to the 1994-1996 birth cohort, and in the 1994-1996 birth cohort relative to the 1989-1993 birth cohort, in analysis of almost 3000 HIV-infected children enrolled in the US-based Pediatric Spectrum of HIV Disease Study (PSD): this was attributed to the increased availability of triple-drug ART regimens[77].

Several classes of ART are now available, each of which targets specific steps in the HIV lifecycle (Figure 1-3). Table 1-2 summarizes some drugs in use today, and the class to which each belongs.

Table 1-2 : Commonly used antiretroviral drugs, by class

ART Class	Drugs
Nucleoside/Nucleotide reverse transcriptase inhibitors (NRTI)	Abacavir, Adefovir, Apricitabine, Didanosine, Emtricitabine, Lamivudine, Stavudine, Tenofovir, Zalcitabine, Zidovudine
Non-nucleoside reverse transcriptase inhibitors (NNRTI)	Delavirdine, Efavirenz, Etravirine, Nevirapine, Rilpivirine
Protease inhibitors (PI)	Amprenavir, Atazanavir, Darunavir, Fosamprenavir, Indinavir, Lopinavir, Nelfinavir, Ritonavir, Saquinavir, Tipranavir
Integrase inhibitors (II)	Raltegravir, Elvitegravir
Fusion inhibitors (FI)	Enfuviritide
Chemokine co-receptor antagonists (CRA)	Maraviroc

Nucleoside reverse transcriptase inhibitors (NRTI) incorporate themselves into the nascent HIV-RNA, produced from the HIV-DNA that entered the host cell from the invading capsid (Figure 1-3), preventing further elongation of the HIV-RNA and prohibiting production of new HIV virions[78-80]. NRTIs are amongst the most widely used antiretroviral drugs, since they were the first available class of drug [81], and also form the "backbone" of many combination regimens[82].

The second class of ART to be developed were the protease inhibitors (PI), which target the viral protease enzyme thus preventing cleavage of precursor virion proteins and so inhibiting formation of new virus particles[83].

Non-nucleoside reverse transcriptase inhibitors (NNRTI) directly inhibit the viral reverse transcriptase enzyme responsible for producing the HIV-DNA from the invader HIV-RNA: NNRTI binds directly to the enzyme and prevents the necessary conformational changes needed to catalyse reverse transcription, ultimately preventing the production of any HIV-DNA[80,84]. The inhibitory effects of NNRTI on HIV were first reported in 1990[85], with the first drug, nevirapine, available in the early 1990s.

Integrase inhibitors (II) target HIV enzyme integrase which is responsible for processing of the nascent HIV-DNA strand produced by viral reverse transcriptase, and integrating it into the host DNA, thus preventing production of new virus particles[86]. Raltegravir was the first II to be approved, in 2007[87].

Fusion inhibitors (FI)[88] and chemokine receptor antagonists (CRA)[89] inhibit binding and entry of HIV into the host cell. The only FI currently available is enfuviritide which must be injected parenterally, making long-term use difficult, especially for children. The first CRA (maraviroc) was licenced in 2007[90].

1.3.4 Combination antiretroviral therapy

Although NRTI mono-therapy was seen to suppress viral load, use of two NRTIs (dual therapy) was more effective[81,91,92]. However, a major breakthrough with respect to HIV treatment came in 1996, with the discovery that a combination of more than two ART drugs was highly effective in preventing disease progression[93-95]. Highly active antiretroviral therapy (HAART) is often used synonymously with the term combination ART (cART). Using more than one drug, or class of drug, results in multiple sites in the HIV replication cycle being targeted, and thus there is a greater likelihood of its interruption. Furthermore, as viral load is suppressed, the chances of treatment failure and emerging drug resistance decrease[96-99].

The *International AIDS Society USA Panel* guidelines recommend that HAART should consist of two NRTIs with either one NNRTI or two PIs (i.e. one PI, plus a booster PI)[100]. The success of HAART in suppressing HIV replication and associated immunosuppression, leading to increased survival, improved quality of life, and reduced morbidity, has been widely reported. In a 1998 investigation, both mono-therapy and non-PI containing regimens were found to be associated with an independent increased risk of morbidity or mortality compared to PI-containing regimens in 1255 patients enrolled in the US-based HIV-Outpatient Study (HOPS): indeed, mortality decreased from 29.4 per 100 person-years in 1995 (the pre-HAART era) to 8.8 per 100 person-years in 1997 (after the introduction of HAART)[62]. Prospective follow-up of 2674 adults enrolled in the Swiss HIV Cohort Study showed that clinical progression was substantially decreased following treatment with HAART compared to the pre-HAART treatment period. This was attributed to increased CD4 cell counts, with a significant increase in hazard of undetectable viral load seen between pre-HAART and post-HAART time periods, i.e. 1995/96 vs. 1997/98 (HR: 1.31, 95% CI: 1.17, 1.48m $p < 0.0001$)[101]. Similarly, in a comparison of two prospective multi-cohort studies, the pre-HAART era Multicentre AIDS Cohort Study (MACS) of 1604 homosexual men first enrolled in the 1980s[102] and the ART-CC study of over 12 000 adults commencing HAART in 1997/8, ART-CC participants had a reduced 3-year probability of AIDS regardless of viral load or CD4+ lymphocyte count[103].

HAART has been shown to be effective in reducing viral load and increasing CD4 cell count in children in both resource-rich[104,105] and resource-poor settings[106,107], although adherence is an important issue[108]. Good adherence has been associated with controlled viraemia in both adults and children. During six month follow-up of 99 adult HIV-infected US-resident patients receiving medication in controlled environments (jail or nursing home), adherence was significantly associated with successful virological outcome ($p < 0.001$), and increase in CD4 lymphocyte count $p = 0.006$) [109]. Poor adherence was significantly associated with virological failure (HIV-RNA ≥ 1000copies/mL) in multivariable analyses of ≤277 HIV-infected adults living in Uganda who were followed up for up to 24 months[110], with poor adherence also reported as a significant and independent risk factor in 4541 subjects enrolled in the Swiss HIV Cohort Study[111]. Thus imperfect adherence is one of the main risk factors for uncontrolled viraemia in treated people[112], with viraemia itself being associated with the emergence of drug resistant strains of HIV[113].

1.3.5 Antiretroviral therapy in children

Specific considerations with respect to treating HIV-infected children include those relating to toxicity, tolerability and formulation. Increasing numbers of drugs are being licenced for use in children[114], but concerns still remain regarding dosage and specific regimen.

Analysis of 1441 children enrolled in the UK National Study of HIV in Pregnancy and Childhood (NSHPC) and Collaborative HIV Paediatric Study (CHIPS) showed not only substantial and sustained decreases in AIDS-associated clinical events since the introduction of HAART, but also increases in CD4% greater than 10%, in children initiating HAART at younger ages and with a lower CD4% at baseline[115]. Longitudinal data from 1142 children enrolled within the Italian Register for HIV infection suggested that triple class therapy was associated with a significant 61% decreased relative hazard of mortality compared to no ART in adjusted models: mono-therapy and dual cART were associated with non-significant hazards of 23% and 30% respectively[95,116]. Moreover, a significant reduced risk of mortality was associated with use of HAART compared to non-HAART regimens (hazard ratio: 0.24, 95% CI: 0.11, 0.51) in a model, adjusted for severity of disease at initiation, using ten-year longitudinal data collected from 1236 children enrolled in a US-based multicentre study[117].

The *Paediatric European Network for the Treatment of AIDS* (PENTA) guidelines[118] explicitly state that treatment in children and adolescents should;

- Achieve and sustain full HIV-RNA suppression with minimization of short term and long term ART drug toxicity.
- Prevent drug resistance.
- Promote normal immune function, thus preventing opportunistic infection.

The United States National Institutes of Health (NIH) guidelines share these aims but with the added proviso that ART should improve the quality of life of paediatric patients[119]. Table 1-3 summarizes the current treatment guidelines published by PENTA, comparing them to the NIH and WHO guidelines.

Commencement of treatment

The Children with Early Antiretroviral Therapy (CHER) trial of 377 South African infants compared immediate ART with deferred treatment (until specific CD4 cell counts or HIV-RNA levels were reached): immediate treatment was associated with a 76% reduction in mortality at age seven months[56]. Furthermore, several observational studies have investigated the optimal time at which to begin ART in HIV-infected infants and children. A significant reduced number of AIDS-related clinical outcomes in children who started ART before three months of age was seen when compared with children who were first treated after three months amongst 210 children in a multicentre European study[120]. Analysis of 131 children enrolled in the ECS showed that both commencement of therapy before 5 months of age, and initiation with HAART were significantly associated with improvements in CD4 cell counts[121]. This is reflected in the recent update to paediatric guidelines[12,122,123], (Table 1-3). Previous guidelines specified clinical, immunological or virological thresholds for starting ART[124]. The durability of first-line HAART commenced before 12 months of age in suppressing viral load and maintaining CD4 counts has been shown during 5 year follow-up of more than 400 children enrolled within the European Pregnancy and Paediatric HIV Cohort Collaboration (EPPICC)[125]. However, the long-term outcomes resulting from ART being initiated at different CD4 counts is unknown.

Table 1-3: Comparison of paediatric treatment guidelines by the Paediatric European Network for the Treatment of AIDS[122], US National Institute of Health [119] and the World Health Organization [126].

	PENTA 2009	US NIH 2010	WHO 2010
0-11 months			
Clinical	Treat all	Treat all	Treat all
Immunological			
Virological			
12-35 months			
Clinical	Treat CDC Stage B or C/WHO stage 3/4	Treat CDC Stage B/C	Treat WHO Stage 3/4
Immunological (CD4%/ count)	Treat<25% or <1000cells/µL	Treat<25%	Treat <25% or <750 cells/µL
Virological	Consider>100 000 copies/mL	Consider>100 000 copies/mL	
12-59 months			
Clinical	Treat CDC Stage B or C/WHO stage 3/4	Treat CDC Stage N/A/specific B conditions	Treat WHO Stage 3/4
Immunological (CD4%/ count)	Treat<20% or <500cells/µL	Treat<25%	Treat<20% or ≤350cells/µL
Virological	Consider>100 000 copies/mL	Consider>100 000 copies/mL	
5+ years			
Clinical	Treat CDC Stage B or C/WHO stage 3/4	Treat CDC Stage N/A/specific B conditions	Treat WHO Stage 3/4
Immunological (CD4% /count)	Treat<350cells/µL	Treat<350cells/µL	Treat<15% or 200cells/µL
Virological	Consider>100 000 copies/mL	Consider>100 000 copies/mL	

PENTA recommend that children aged over 12 years be treated with ART according to specified thresholds (Table 1-3). There is considerable debate regarding whether it is more appropriate to defer treatment in children (i.e. those aged >12 months) as opposed to adults[127]. It has been argued that due to the absence of randomized clinical trials in this age group, the evidence supporting early treatment with ART is incomplete. Furthermore, as treatment in children is likely to be life-long, children are more likely to accumulate decades of ART, and the effects of this are unknown: in this scenario, a potential approach to minimize cumulative exposure is to defer treatment. However, childhood mortality correlates with poor clinical status (including increased viral load) at the commencement of treatment in infancy and early childhood[128]: once HIV-infected children survive to 1 year of age, the rate of disease progression is relatively slow indicating the importance of maintaining health a young ages.

The economic implications of ART have been highlighted[129,130], and may be a consideration in deciding on timing of treatment. In certain contexts, for example low-income countries, decisions may therefore be made to "ration"[131] treatment until clinical symptoms appear or acceptable CD4 levels reached, rather than adopting the recommendation of early treatment.

Adherence

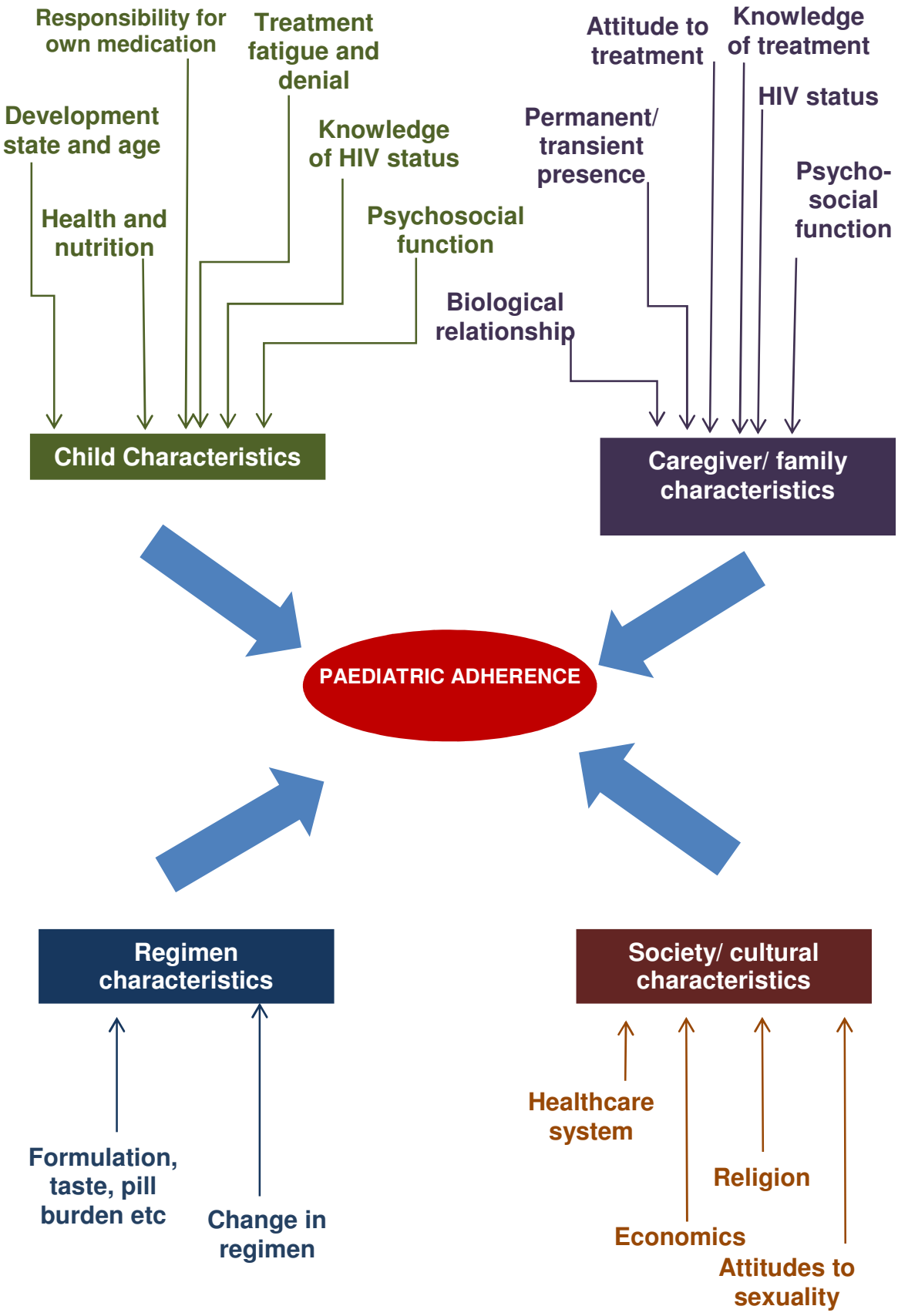
The availability of ART formulations which are palatable to children are still comparatively rare, which has contributed to lower rates of adherence in children[132,133]. Both the tolerability and toxicity of specific ART in children and adolescents has been well documented[134,135]: this may

influence patient adherence to treatment. The desire to avoid both the direct effect of toxicity in paediatric patients, and also the indirect effects (such as poor adherence leading to viral rebound, AIDS-related clinical symptoms, and the emergence of drug-resistant HIV strains[136-138]) may also contribute to the argument against early treatment.

The relationship between adherence to ART and the emergence of drug resistance is in itself complex[139,140], with evidence suggesting that resistant strains can occur at high levels of adherence to non-ritonavir boosted PI-based ART, but at poor-to-moderate levels of adherence to NNRTI-based ART[141,142], reflecting the long half-life of NNRTI[143]. Relatively high levels of suboptimal adherence to HAART can occur before clinical effects are seen: during 12-month follow-up of 195 adult patients with initial undetectable viral load, HAART adherence of 70-89% was associated with viral rebound with clinically significant resistance[144].

Poor adherence is associated with viral rebound in children[145,146], but few studies have investigated associations between adherence and clinical outcome in childhood. Good adherence in younger children requires their co-operation in the administration of medication, but is also complicated by the important role of the care giver or parent. This individual must have an understanding of the treatment, a commitment to the health of the child, organized to give the drugs at the appropriate time, and (in some cases), the economic ability to provide medication[147]. Furthermore, late adolescence where management of treatment moves from adult carers to the teenager can be a difficult time in terms of the maintenance of adequate drug adherence. In addition attitudes in the society may be important. For example disclosure of HIV status may be an issue in most communities where there is prejudice against HIV: adherence may be associated with a risk of disclosure in adolescents, especially where drugs need to be taken during the working day[148]. The factors involved in paediatric ART adherence are illustrated in Figure 1-4.

Figure 1-4: Factors associated with paediatric and adolescent adherence to antiretroviral therapy (adapted from Haberer and Mellins (2009)[146])



A systematic review of 17 paediatric studies found that rates of adherence ranged from 49% to 100%, with over three-quarters of studies reporting rates of over 75%[149]. Furthermore meta-analysis estimates of adult adherence were 55% in North America and 77% in Africa[150]. A direct comparison of adolescents (aged 11-19 years) and adults in a South African population showed that adolescents were less adherent, with lower rates of virological suppression and immunological recovery ($p < 0.01$)[151].

Switching ART regimen

Change in ART regimens to maintain HIV viral suppression has been examined in large prospective studies[152], in part prompted by the observation that the rate of virological rebound in adults treated with either PI-based ART or HAART was as high as 40% in the late 1990s[101,153]. In a recent Ugandan-based prospective study of 124 children initiating HAART, 14% (n = 17) displayed virological failure (HIV-DNA ≥400 copies/mL) after 48 weeks of treatment, 3 of whom were infants aged <1 year[154]. In a Cambodian study following 212 children commencing HAART, 19% had virological failure following at least one year of treatment[155]. Other paediatric studies have reported similar results[156,157]. Thus it is likely that children and adolescents may be subject to increasing ART switching between drugs and regimens through their lifetimes in order to maintain viral suppression.

Age at initiation of ART is an important factor in HIV-suppression, and thus also an important factor in switching of regimens. In analysis of 324 ART-naïve HIV-infected children enrolled in CHIPS, CD4 cell increases were more likely in younger children, but viral load suppression was more likely in older children ($p < 0.001$ for both). Furthermore, long term increases in CD4% occurred faster, and decreases in viral load slower in younger children (follow-up of 6 months versus 24 months): these results were all independent of pre-treatment CD4 and viral load status[158]. The poorer virological outcome with increasing age may result in switching of regimens to maintain suppression. Concurrently, the durability of first-line regimens has improved over calendar time. EPPICC reported an increased proportion of subjects with viral suppression between 1996-1999 and 2004-2008: rates of switching to second-line regimens were lower among children starting four-drug NNRTI-based and boosted-PI regimens, and two-thirds of subjects remained on first-line regimens for over five years (analysis of 426 children followed up for a median of 5.9 years after the commencement of ART)[125]. Thus switching to second and third line regimens may be deferred due to success in maintaining HIV suppression on current treatment.

The aim of the PENPACT-1[d] clinical trial of 266 children was to compare strategies of starting and changing ART on long-term virological suppression in ART-naïve children. Results suggested that switching may have consequences in terms of resistance, dependent on regimen and indicators for switching. The trial investigated the effectiveness of PI-based and NNRTI-based HAART, and switching to second-line ART of these regimens at high and low viral load thresholds by randomizing participants to four arms: switching at higher viral load resulted in accumulation of more NRTI resistance mutations compared to the lower threshold in the NNRTI arms, while there was no difference in NRTI or PI mutations in the PI arms regardless of the viral load at switching[159]. Thus despite the recent studies looking at switching paediatric populations the long-term effects of switching treatments in children are still unknown. Nevertheless, PENPACT-1 reported that over 70% of children remained on first-line regimens at 5 years following initiation of treatment, reiterating the increased stability of these drugs in recent years.

Results from the PLATO II (Pursuing Later Treatment Options II) study of 1007 HIV-infected children demonstrated the rate of triple class (ART regimen containing at least one each of PI, NNRTI and NRTI) failure and associated factors over an average follow-up of 4 years: while the observed rate of 12% was relatively low, it was twice that of adults[160]. Thus, there are still substantial challenges in maintaining durable viral suppression in children which is exacerbated by both the limited number of ART formulations available for children, and the need to provide life-long treatment.

Vertical transmission of HIV and perinatal antiretroviral treatment

Most infections occur in children as a result of vertical transmission (MTCT), which may occur before, during or following birth[161-163]. Combined analysis of data from three Africa-based studies and one Australia-based studies estimated a 29% risk of transmission of HIV-1 from postnatally infected mothers to their children through breast milk[164]. In a Kenyan-based 24-month prospective clinical trial, transmission rates were significantly higher in breast-fed versus formula-fed infants[165]. However, the risk of transmission associated with exclusive breast feeding compared to non-exclusive breast feeding is controversial[166]

Effective options for preventing vertical transmission other than avoiding breastfeeding include avoidance of infant exposure to contaminated maternal secretions by elective caesarean section delivery[167]. Indeed, rates of caesarean sections in HIV-infected pregnant women enrolled in the multicentre ECS have been seen to increase steadily[168] since early reports that vaginal mode of delivery was associated with a (non-significant) increase in vertical transmission[169].

[d] Paediatric European Network for Treatment of AIDS (PENTA) and Pediatric AIDS Clinical Trials Group (PACTG/IMPAACT)

Analysis of 19, 494 HIV-infected women resident in South Africa and who had recently given birth showed significant protective effects against vertical transmission associated with ART use in pregnancy, or lifelong maternal ART use[170]. Specific ART during pregnancy was shown to have a significant protective effect against vertical transmission (compared to zidovudine mono-therapy) in a Brazilian study of 452 women[171]. Postpartum ART in babies born to HIV-infected mothers who have either received no treatment during pregnancy[172] or presented for delivery late with unknown HIV-status[173] has been shown to result in reduced rates of infection in the weeks following birth. Other studies have found reduced rates of vertical transmission following intrapartum and/or postpartum ART in infants who were/were not breastfed[174-176]. Furthermore, ART treatment during late pregnancy followed by postpartum treatment in the infant has been shown to reduce the risk of postnatal infection at 24 months[177]. A Cochrane review has concluded that reductions in vertical transmission can be made by avoidance of breast feeding by HIV-infected mothers, or in the case of breast feeding occurring: exclusive breast feeding during the first few months of life, and/or chronic antiretroviral prophylaxis in the infant[178] Indeed rates of vertical transmission have decreased over time since the introduction of HAART treatment[179].

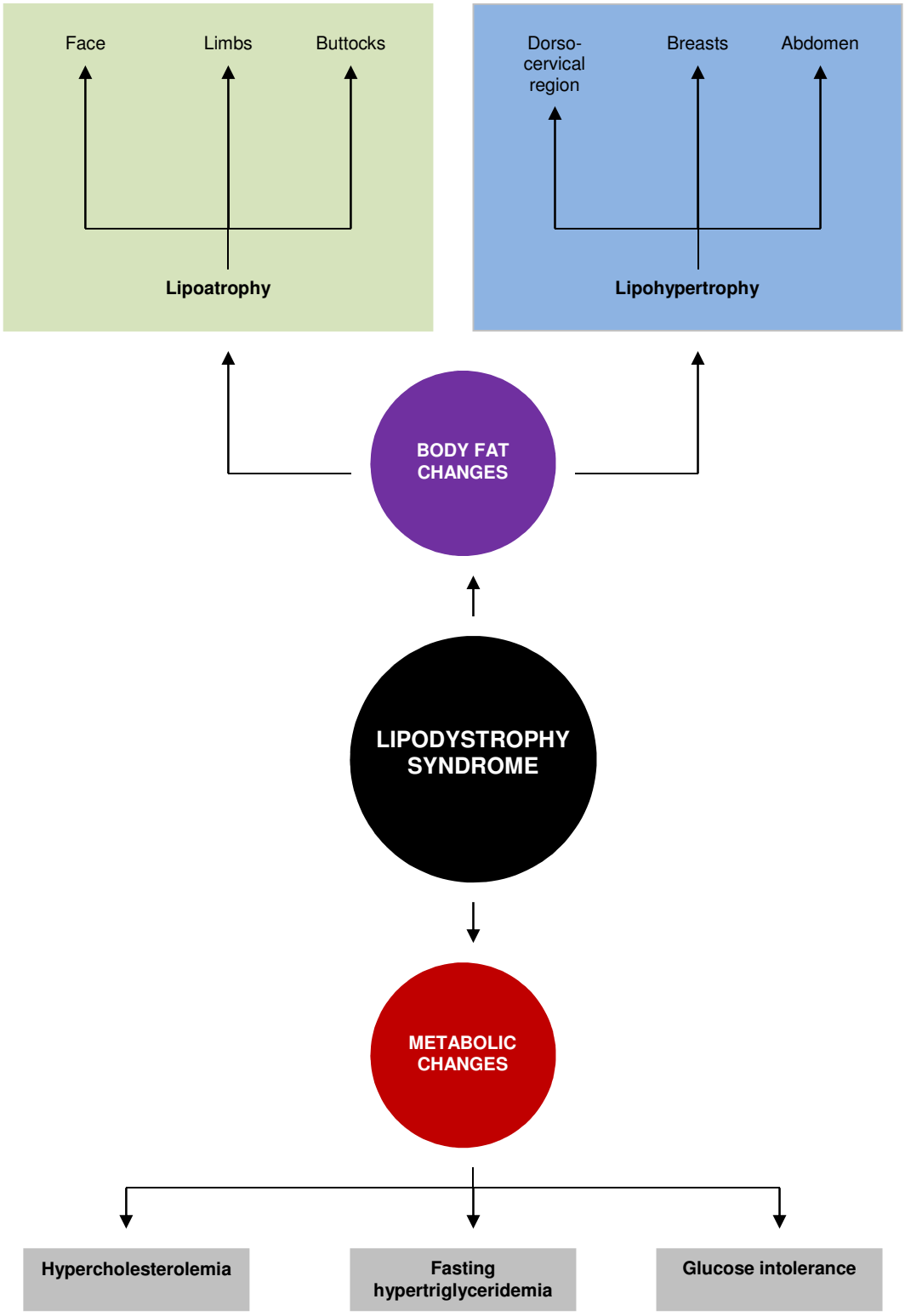
Social and economic pressures may limit the uptake of initiatives aimed at reducing vertical transmission through reduced rates of breast feeding and/or prophylactic treatment during pregnancy[180-182]. This may explain why the percentage of HIV-infected pregnant women receiving prophylactic ART is higher in Europe and Central Asia compared to Sub-Saharan Africa[13]. Indeed, the mother-to-child transmission rate in the UK has been reported as being less than 2%, being reduced further in women who have received at least 14 days of ART[183].

1.4 Lipodystrophy syndrome

1.4.1 Phenotype

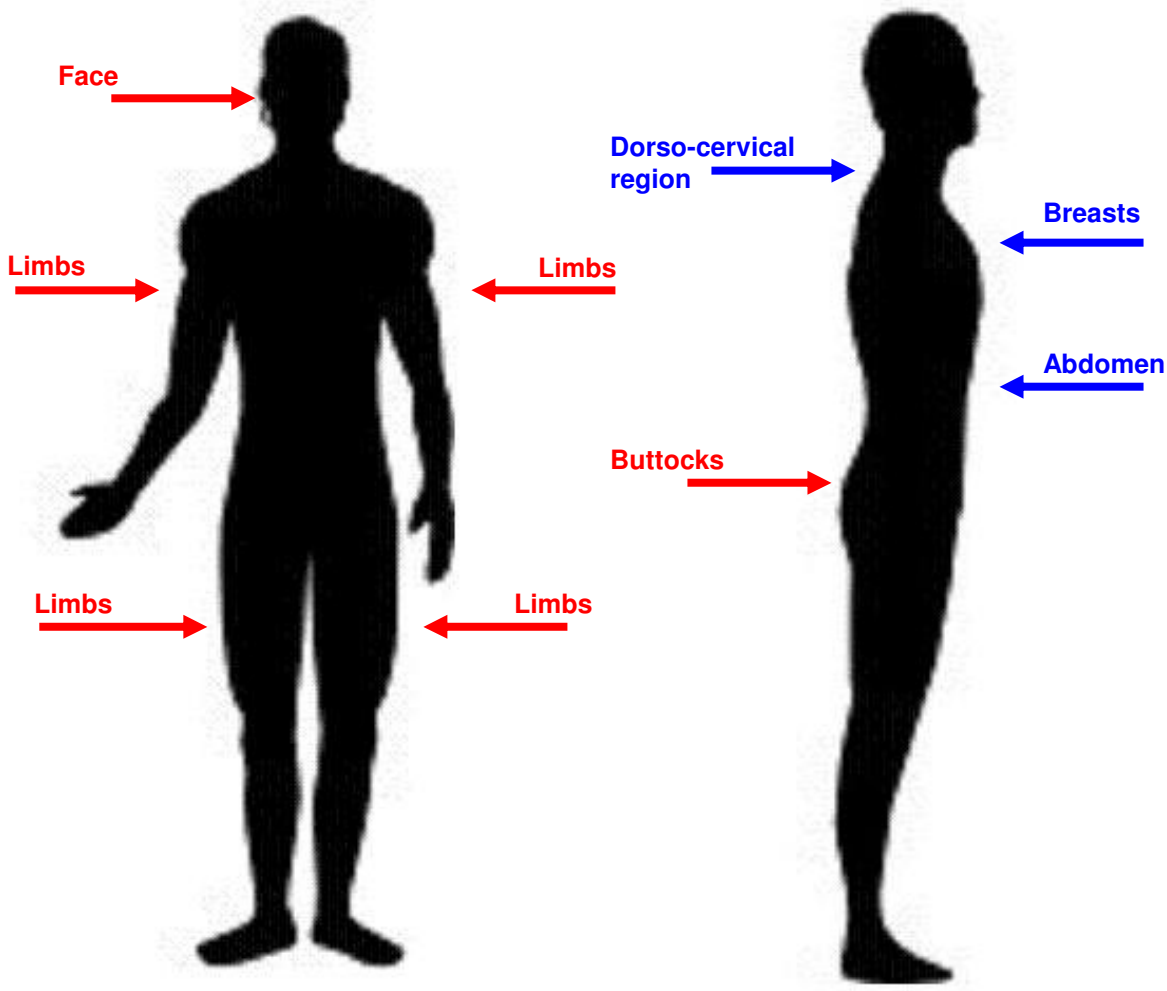
Lipodystrophy syndrome (LS) can be defined as a body fat alteration which is usually accompanied by metabolic abnormality. The body fat alteration may manifest as lipoatrophy (fat loss occurring in at least one of the following: face, limbs or buttocks) and/or as lipohypertrophy (fat gain occurring in at least one of the following: back of neck, breasts or abdomen). The metabolic abnormality can take the form of hypercholesterolemia, fasting hypertriglyceridemia or glucose intolerance. Figure 1-5 illustrates this definition.

Figure 1-5: Definition of lipodystrophy syndrome incorporates body fat alterations at distinct body locations, and specific metabolic abnormalities



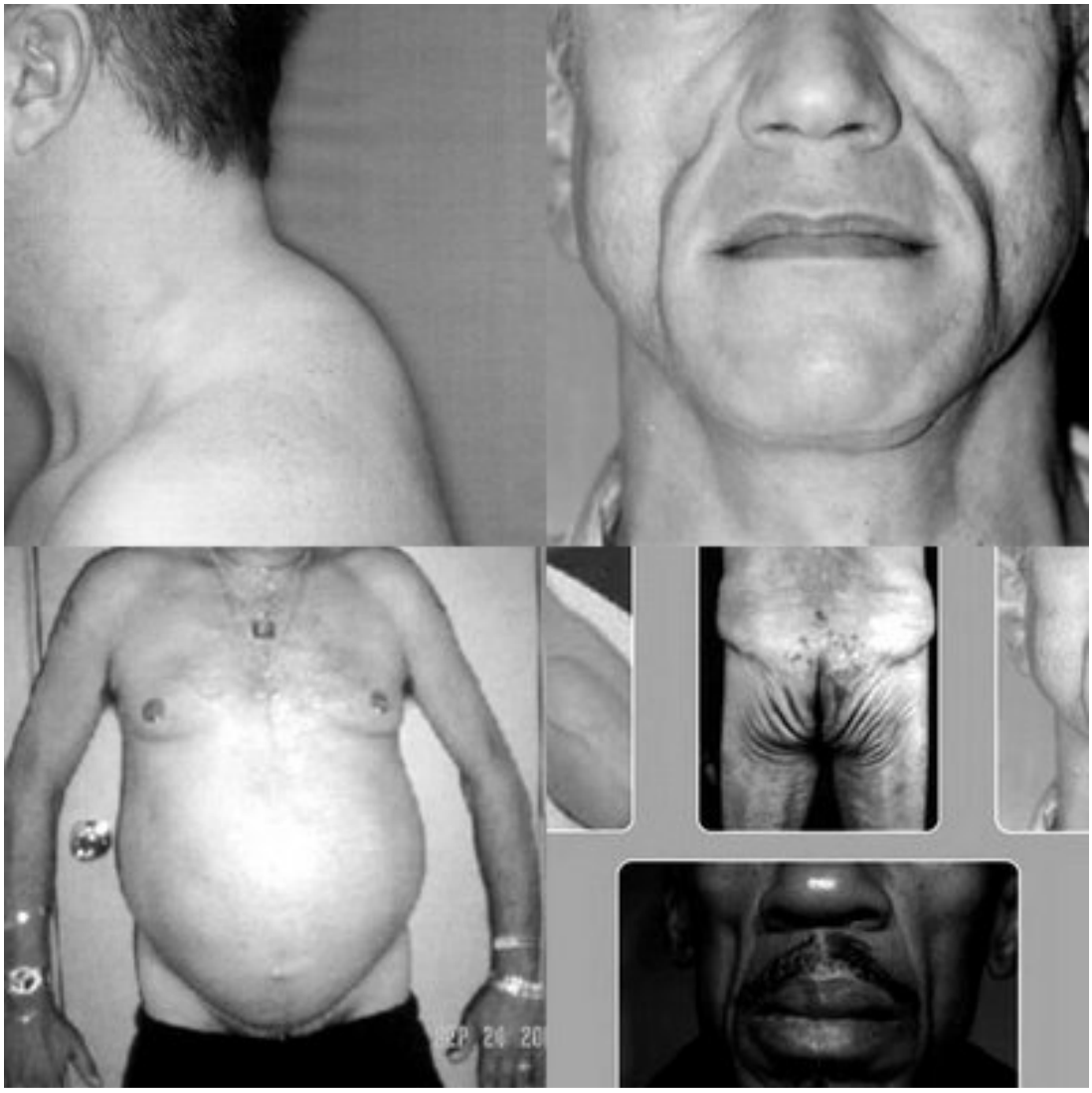
The body fat alterations can take the form of either lipoatrophy in the face, arms, buttocks or limbs, or lipohypertrophy in the back/neck ('buffalo hump'), breasts or abdomen, as illustrated schematically in Figure1-6, and photographically in adult (Figure1-7) and paediatric (Figure 1-8) patients.

Figure 1-6: Body fat alterations seen in lipodystrophy syndrome (LS) by location.



Areas susceptible to lipoatrophy are shown in red and locations of lipohypertrophy are shown in blue.

Figure 1-7: Manifestation of body fat alterations seen in male adults with lipodystrophy syndrome.



Top left; dorso-cervical lipohypertrophy (buffalo hump), bottom left; abdominal lipohypertrophy, top/bottom right; face lipoatrophy, and middle left; buttock lipoatrophy. Retrieved from http://survivinghiv.blogspot.com

Figure 1-8: Manifestation of body fat alterations seen in paediatric patients with lipodystrophy syndrome.

Left; 20 year old male with breast lipohypertrophy, and right; 15 year old female with dorso-cervical lipohypertrophy (buffalo hump). Reproduced courtesy of Dr. Alex Peltier.

LS may occur as either fat alteration without metabolic abnormality, or metabolic abnormality without fat alteration. With different combinations of fat disorder and metabolic components, the phenotype becomes more heterogeneous. This has led to the argument that LS can be divided into three, possibly overlapping, conditions[184]:

1. Lipoatrophy: subcutaneous fat loss (especially affecting the limbs, face and buttocks), and deep fat loss (visible at the level of "Bichat", pre-auricular or orbital fat pads, and manifesting in pain triggered by mastication, sunken eyeballs and retro-orbital pain). This may be associated with an increase in triglyceride levels and insulin levels, but remaining in the normal range.
2. Mixed syndrome: peripheral fat loss (limbs, buttocks, face), and fat gain, but no deep fat loss. This may be associated with metabolic changes similar to those seen in insulin resistance, i.e. raised insulin, C-peptide, and free fatty acid.
3. Subcutaneous adiposity: this may manifest with fat accumulation in the dorso-cervical and breast region, but not seen to the same extent as seen in obesity. Furthermore, there is no increase in visceral adipose tissue area, nor any insulin resistance.

However, this may be an over simplification as other phenotypes not easily categorized within this classification have been observed Furthermore, the described categories above were proposed from cross-sectional analysis of 154 male adults. In a US study comparing 30 HIV-infected adults to non-infected controls, significant differences in mean triglycerides and high density lipoprotein (HDL) cholesterol were seen between patients with lipohypertrophy and

those with both lipohypertrophy and lipoatrophy: thus demonstrating a syndrome of insulin resistance characterized by hyperinsulinaemia, hypercholesterolemia, low HDL, and truncal lipohypertrophy[185]. Moreover, prospective follow-up of patients who started on different ART in a US study (n = 41) found PI use to be significantly associated with increases in insulin, glucose, triglyceride, low density lipoprotein (LDL) cholesterol and total cholesterol, but these changes were independent of fat alterations[186].

The problematic phenotype characterization of LS is compounded by an incomplete understanding of the relationship between metabolic abnormality and fat alterations, particularly with respect to temporality. The relationship between lipoatrophy and lipohypertrophy is complex. It is thought that the inflammatory sequelae of both HIV infection and ART reduce the capacity of adipose cells to store lipid, and also cause the release of free fatty acid through increased lipolysis and adipocyte apoptosis, ultimately resulting in lipoatrophy[187]. Indeed, it has been argued that distinct adipocyte depots within the body react to PIs differently, with subcutaneous adipocytes more predisposed to becoming insulin resistant and lipoatrophy processes[188,189]. In contrast, the pathogenesis of lipohypertrophy is less well understood, possibly occurring secondary to lipoatrophy as a consequence of fat no longer being able to be stored in subcutaneous tissue[187]. However, this proposed pathogenesis does not address the situation where lipohypertrophy occurs in the absence of lipoatrophy.

1.4.2 Prevalence

LS was first described in HIV-infected adults undergoing ART in 1997[190], but has also been described in treatment naïve patients[191]. The timing of the recognition of ART-associated LS reflects the accumulating exposure to ART in the treated population and the emerging use of HAART[8]. Prevalence in HIV-infected adults of fat alterations and/or metabolic abnormality was 50% in a 1999 Canadian population receiving ART[192], and of fat alterations of 70% in a 2006 Rwandan population receiving HAART[193].

Fewer studies have concentrated on the epidemiology of LS in children and adolescents than in adults. The syndrome was first described in children in a French study in 2000; 13 individuals from a sample of 33 HIV-infected children (mean age 9 years) showed evidence of body fat changes and also exhibited non-significant hypertriglyceridemia compared with children with no body fat changes[194]. In another early, cross–sectional, study of 40 HIV-infected children (mean age of 9.1 years), the prevalence of fat alterations was 18%, and of hypertriglyceridemia and/or hypercholesterolemia was 73%. Although no significant associations between postulated risk factors and LS were found, children receiving paediatric dosing regimens were less likely to develop LS in comparison to children on adult regimens (p = 0.0003)[195]; however, this association may have been confounded with age and pubertal stage of participants. Both the largest paediatric studies into LS, with 1812 and 2122 participants were nested within the same

prospective cohort, the Paediatric AIDS Clinical Trials Group 219c (PACT 219C): they reported a prevalence of hypercholesterolemia (defined by age, race and gender cut-offs) of 13% (95% CI: 11.1, 14.3 among 229 HIV-infected children)[196], with an incidence of 13% (defined as cholesterol >220mg/dL) in 2122 susceptible subjects over a median follow-up of 50.4 months (incidence rate; 3.4 cases per 100 person-years, 95% CI: 3.0, 3.9)[197]

The lack of consensus regarding the definition of the LS phenotype makes it difficult to establish valid comparisons between reported prevalence and incidence from different studies. Furthermore, there are comparatively few prospective studies with follow-up of large numbers of participants. In a 24 month prospective study of 84 HIV-infected adults treated with HAART enrolled in a French cohort, LS was defined as at least one of the following; abdominal lipohypertrophy, fat lipoatrophy, limb wasting, breast lipohypertrophy, pleomegalia, or alopecia: the incidence of LS was reported as 5% at 6 months following treatment initiation, increasing to 26% at 9 months and remaining at his level at 12 and 24 months post initiation[198]. A similar Spanish study of 496 ART-naïve adult patients commencing HAART and followed-up over 24 months defined LS as central obesity and/or subcutaneous lipoatrophy: the incidence was reported as being 11.7 per 100 person/years (95% CI; 9.2, 14.2), i.e. 17% of the cohort[199].

Similar problems regarding herterogeneity of outcome are seen in published paediatric research: some studies looked at fat alterations as a whole[195,200-208], while others specifically examined lipoatrophy or lipohypertrophy[194,209,210]. While most studies in children investigated clinically-assessed changes in body shape, others also used measures such as skin fold thickness[211] or estimation of percentage body fat[212,213]. The most recent study to investigate body fat alterations in HIV-infected children reported a prevalence of 25.0% (95% CI: 14.8, 34.6): this was a cross-sectional study of 57 children (median age 9.5 years) living in Spain with alterations assessed by the treating clinician[207]. In contrast, a French cross-sectional study of 39 children (median age: 9.1 years) reported a prevalence of body fat alterations of 33.3%: peripheral fat wasting was defined as face/buttock/limb atrophy with arm skinfold thickness <3rd percentile of reference values for sex and age in a French population, and trunk adiposity defined as breast enlargement/back of neck/abdominal enlargement with trunk:arm skinfold ratio >2 standard deviations from the mean[194]. As with adults most paediatric studies follow a cross-sectional design which precludes the estimation of incidence. Furthermore, excluding the PACTG 219C study, the largest of the longitudinal studies contained 178 participants[200,201,203,204,210,211]. Although valuable prevalence and descriptive data has been collected in cross sectional studies, advances in understanding the pathogenesis of LS in children and adolescents require longitudinal analyses of both existing and incident cases. Ideally these studies should be prospective in nature as retrospective studies are hampered by data collection from secondary sources, such as patient notes, rather than utilizing standardized and focused tools

A review of adult studies estimating the prevalence of LS, defined as any sign of fat alterations, reported a range of prevalence between 2% and 84%. The heterogeneity in these estimates was attributed not only to varied definitions of the syndrome, but also a mixture of self-reported and clinical methods of assessment[214]. HOPS, a cohort which enrolled HIV-infected adults in eight US studies, reported a prevalence of fat alterations of 49% among 1080 patients[215], while the prevalence of fat alterations was 38% and of metabolic abnormality was 49% amongst the 581 patients from five hospitals enrolled in the Aquitaine Cohort[216]. The largest cross-sectional study in paediatric LS, the European Paediatric Lipodystrophy Cohort multi-centre investigation including 477 children of median age 9.8 years, reported a prevalence of body fat alterations of 26%, of hypercholesterolemia of 21% and of hypertriglyceridemia of 27%[209].

There is evidence that body fat disorders occurring in HIV infected children can progress over time. Body fat mass was measured using dual energy x-ray absorptiometry (DEXA) in a prospective observational study of 28 pre-pubertal HIV-infected children aged 4 -13 years with an average of 1.2 years follow-up. Eight children were assessed to have limb lipoatrophy and truncal lipohypertrophy at baseline, and there was significant decrease in body fat percentage during follow-up in these children ($p < 0.05$). In addition, non-significantly decreased limb fat over time was seen in children with initial fat disorder, but a significant increase in limb fat was seen in children without initial fat disorder ($p \leq 0.01$)[200]. However, this study had low statistical power and thus independent associations with risk factors, such as ART use, could not be assessed.

1.4.3 Pathogenesis

Both HIV- and ART-dependent pathways of LS pathogenesis have been proposed.

HIV and lipodystrophy syndrome

An ART-independent mechanism of pathogenesis of LS remains controversial. Although studies have found independent associations of LS with duration of HIV infection (patients with LS having longer durations of infection compared to patient without[217]), CD4 cell count (patients with LS being shown to have lower CD4 cell counts than patients without[200]), and viral load (patients with LS having lower viral load compared to patients without[218]), the role of these factors in pathogenesis is unclear. Alterations in adipose gene expression have been observed in treatment naïve HIV-infected patients[219], providing evidence of ART-independent pathways of lipodystrophy pathogenesis. A relationship between HIV-RNA and resting energy expenditure has been reported[220], suggesting that the host immune response may play a role in lipoatrophy by increasing energy demand when activated, and ultimately resulting in the breakdown of energy stores such as adipose tissue. Tumour necrosis factor alpha (TNF) [221] and interleukin (IL)-6 [222] levels are increased in subcutaneous adipocytes from lipoatrophic patients and it is thought that the infection-stimulated pro-inflammatory cytokines TNF, IL-1, and IL-2 have a role in stimulating hyper-metabolism and protein turnover.

Association between lipodystrophy syndrome and antiretroviral therapy

Studies have found NRTIs, PIs and NNRTIs all to be associated with increased risk of LS in adults[223-227]. In a cross-sectional study of HIV-infected adult women, fat alteration was associated with long-term antiretroviral use, with a significant correlation with use of lamivudine. Furthermore, PIs were associated with higher risk of developing fat alterations, although this was also seen in patients on non-PI regimens[228].

Investigation of body fat changes in a paediatric population can be blurred by the rapid growth and development that is characteristic of childhood and adolescence. This is further complicated by the fact that poor growth in HIV-infected children is well recognized[200,229,230]. Thus it may be difficult to separate these effects from the changes in body shape mediated by ART, especially as ART has been shown to improve growth[231]. The concurrent effects of natural growth, HIV-infection and ART may manifest in a dynamic of progression and regression of lipodystrophy which may not be seen in adults.

Paediatric studies have reported significant increased risk of body fat alterations associated with both PI and NRTI use[205,209]. DEXA analysis of body fat tissue in children in Italy found a decreasing trend in the ratio of trunk fat to total fat, and an increasing trend in the ratio of limb fat to total fat, across HIV-infected children with LS, HIV-infected children without LS and HIV-uninfected matched controls[232], indicating similar patterns of fat alterations as seen in adults. Further analysis of 37 HIV-infected children (mean age, 12.2 years at baseline) from this study population indicated that both trunk lipohypertrophy and limb lipoatrophy are features of LS in children. Significant ($p < 0.01$) decreases in arm and limb fat over 12 months follow-up was reported. There were also significant ($p < 0.05$) associations of limb/lean muscle ratio with pubertal stage, sex, increasing body mass index (BMI) and duration of PI-based HAART therapy in multivariate models controlling for age and past and current immunosuppression. Pubertal stage and duration of PI-based HAART were found to be independent predictors of truncal adipose tissue, with a negative association seen in multivariable modelling[233].

The use and duration of use of indinavir were associated with the accumulation of intra-abdominal fat in a US-based cross-sectional analysis of 30 HIV-infected adult males[234]. Progressive longitudinal monitoring of fat wasting in HIV patients on different drug regimens suggests that NRTIs are associated with lipoatrophy, with PIs having a synergistic effect[235]. In a prospective study (median follow-up time of 3.1 years) investigating the effect of different NRTIs in the presence of similar PI-based regimens, it was found that the different NRTI drugs were associated with varied fat disorder outcomes: stavudine and zidovudine were associated with decreases in subcutaneous fat in the mid-arm, mid-thigh and waist, while lamivudine was associated with increased subcutaneous adipose tissue in the same locations and waist non-subcutaneous tissue[236]. Furthermore, an investigation into the effect of switching drug regimens

concluded that NRTIs, particularly thymidine analogues such as zidovudine and stavudine, are particularly associated with lipoatrophy in the extremities[237]. The role of thymidine analogues in lipoatrophy is documented by other studies[238]: however less toxicity is reported with newer drugs such as tenofovir and abacavir[239]

Total cholesterol and LDL cholesterol was significantly higher in HIV-infected patients with LS (*n* = 10) compared to those without LS in a US-based cross-sectional study[240]. A US-based historic cohort which followed-up of 221 HIV-infected adults over 5 years reported that the cumulative incidence of hypercholesterolemia, hypertriglyceridemia and body fat alterations increased after the initiation of PI therapy, with increased triglyceride levels significantly associated with PI use, and increased cholesterol levels significantly associated with months of both PI use and NRTI use[241]. Furthermore switching from PI to nevirapine in treatment regiments resulted in significant reduced concentrations of cholesterol and triglyceride in 23 treated HIV-infected adults[242]. Indeed, there were significant increases in median total cholesterol, LDL-cholesterol and HDL cholesterol in 48 HAART-naïve HIV-infected adults who commenced PI-based HAART in a US-based study[243].

Paediatric studies have also investigated metabolic abnormality in children on ART[201,203,208].. In a UK-based cross-sectional study, using untreated controls, significantly increased total cholesterol levels were found in children on HAART with PIs (*n* = 24) and in those on non-PI HAART (*n* = 35) compared to untreated children (n=39). Furthermore, there were significant differences in several anthropometric measures between children on PI-containing HAART compared with untreated children, but such differences were not significant when comparing non-PI HAART-treated children and untreated children[244]. During 48-week follow-up of 50 previously PI-naïve HIV-infected children in a Thai study, significant increases were seen in total cholesterol, LDL cholesterol, and triglyceride levels[245]. Furthermore, 48-week prospective follow-up of 28 HIV-infected children (aged 5-17.5 years) who were switched from PI to efavirenz (and from stavudine to tenofovir) resulted in significant decreases in levels of total cholesterol, LDL-cholesterol and triglyceride[246]. During 96-week prospective follow-up of 20 HIV-infected children (median age 7.9 years) who had previously been on PI-containing regimens for >4 years, switching to a triple NRTI-based strategy resulted in significant decreases in levels of total cholesterol and triglyceride[247]. Indeed, several studies have reported hyperlipidemia to be associated with PI use in HIV-infected children[248-250]. In cross-sectional analysis of 386 HIV-infected children (7-24 years old), not only were significant predicted increases in mean total cholesterol, LDL-cholesterol, non-HDL cholesterol, and triglyceride seen to be associated with PI use (ritonavir and nelfinavir) [251], but the same study also reported opposite effects on HDL-cholesterol significantly associated with different NRTIs (decrease with zidovudine and increase with nevirapine)[251]. Furthermore, research in HIV-infected adults has suggested that NNRTI use may be associated with a significant and independent protective effect against low HDL-cholesterol[252].

The role of ART as a key risk factor for LS in children is reinforced by results from studies that have investigated both body fat alterations and metabolic abnormality. The observational European Paediatric Lipodystrophy Group study of 477 HIV-infected children (median age 9.8 years) found a prevalence of 26% for any fat alterations, with lipohypertrophy in the trunk and neck, and lipoatrophy in the face and limbs occurring more commonly than fat alterations in other locations. In adjusted analyses, increased risk of fat alterations was independently associated with ever use of PIs and ever use of stavudine. Prevalence of dyslipidemia in the study group was 38%, with significant 2-fold and 5-fold increased risk of hypercholesterolemia with gender and ever-use of PIs respectively (following adjustment for current ART use and lipohypertrophy). Ever-use of stavudine was associated with a significant 4-fold, and ever-use of ritonavir was associated with a significant 2-fold, increased risk of hypertriglyceridemia, after adjustment for lipoatrophy and CDC clinical status[209]. In a North American cross-sectional study of 386 HIV-infected children aged 7-24 years, mean total fat and limb adiposity were lower in HIV-infected subjects compared non-HIV infected controls, with trunk adiposity being lower in HIV-infected children on PI-therapy compared to those not on PI therapy (adiposity assessed using DEXA). Similarly, median triglyceride levels were highest in the HIV-infected group treated with PIs compared to the HIV-infected PI-naïve group and the uninfected controls[251]. In a Canadian study of 48 subjects, insulin sensitivity in PI-treated children was significantly lower than that seen in PI-naïve children, following adjustment for potential confounding variables, suggesting that LS-related insulin resistance also occurs in children. Central adipose tissue area, and the ratio of central to subcutaneous adipose tissue were non-significantly higher in PI-treated children compared to PI-naïve children[253].

Postulated mechanisms for antiretroviral therapy-mediated lipodystrophy

Several mechanisms of PI-mediated lipodystrophy have been proposed[254]. Elevated plasma glucose may be mediated by the effect of PIs on hepatic glucose production[255,256] and also glucose disposal[257]. This may occur in several ways. PIs[258,259] including ritonavir [260] have been shown to reduced insulin-stimulated glucose uptake by the Glucose Transporter Type 4 (GLUT-4) in rat adipocytes. Cytochrome P-450 enzymes, which are central to lipid metabolism[261], are inhibited by PIs[83]. Animal, particularly murine, models have shown that ritonavir induces hyperlipidemia by (a) reducing the activity of the enzyme hepatic lipase[262] thus impeding hydrolysis of circulating triglycerides and the cellular uptake of lipoproteins and lipids[263], and (b) suppressing the down regulation of sterol regulatory protein (SREBP) an adipose-inducing lipid metabolism protein[264].

Direct effects on adipocyte differentiation and apoptosis (resulting in reductions in chylomicron uptake, triglyceride clearance and adiponectin release) may be associated with several PI-induced pathways; increased release of inflammatory cytokine levels (TNF-α, IL-6, IL-1β); inhibited cytoplasmic retinoic acid binding protein 1 (CRABP-1); and inhibited lipoprotein receptor related protein (LRP) [221,255,265-267]. Increased rates of triglyceride lipolysis are then

responsible for elevated plasma free fatty acids, including LDL cholesterol and very low density lipoprotein (VLDL) cholesterol[268-271]. This metabolic abnormality may therefore lead to changes in insulin sensitivity, and ultimately to a state of insulin resistance, particularly in the muscle and liver [272].

The mitochondrial toxicity of NRTIs has been widely investigated[273]. Indeed, the effect of NRTI-containing ART regimen on mitochondria in infants and children has been noted[274,275]. The lipodystrophy-promoting effects of these drugs may be linked to this toxicity[276], with several features of NRTI mitochondrial toxicity, e.g. polyneuropathy, hepatic stenosis and hyperlactatemia occurring in HAART-associated LS[277] (although these may in fact be occurring concurrently with LS). Mitochondrial dysfunction may result in lipohypertrophy through a reduction of the mitochondrial enzyme cytochrome oxidase, and impaired beta-oxidation of fatty acids resulting in accumulation of fat droplets within cells[278].

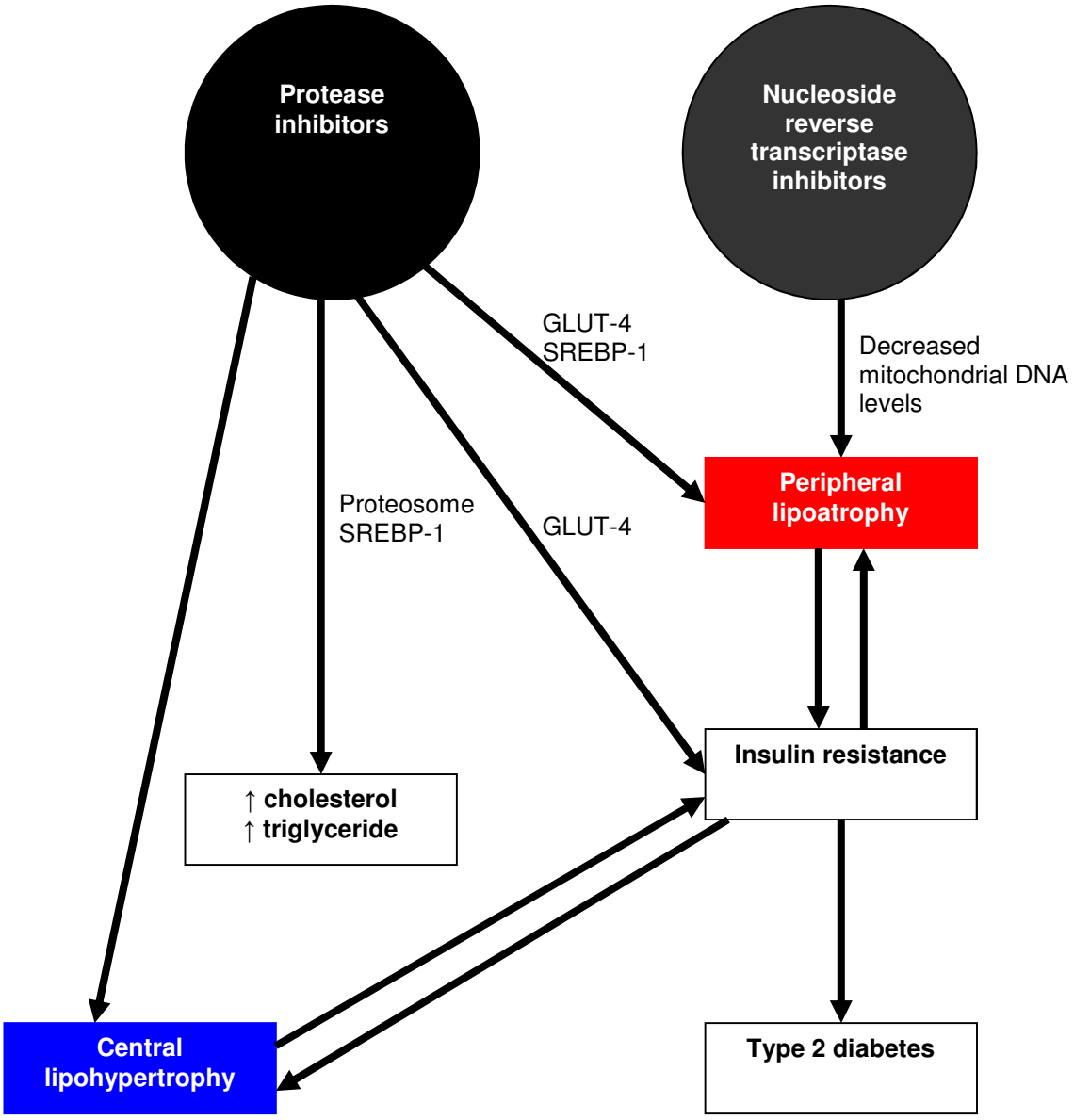
Mitochondrial toxicity is also implicated in lipoatrophy because of the observation of abdominal distension and lactic acidosis in lipoatrophic patients. The mechanisms postulated have included mutations in mitochondrial DNA polymerase (as seen in similar syndromes such as Multiple Symmetric Lipomatosis), or impairment of hepatic glycogen and fat oxidation resulting in increased oxidation of peripheral energy stores inducing lipoatrophy[277]. Levels of mitochondrial DNA (mtDNA) have been found to be lower in subcutaneous tissue in patients on NRTI compared to patients on either other regimes or who were ART naïve[279,280]. Depletion of mtDNA leads to disturbances in the balance of oxidative phosphorylation and can result in lactic acidaemia/acidosis[281]. It has also been suggested that the NRTI-mediated inhibition of mtDNA polymerase results in depletion of mtDNA in adipose tissue to below the threshold at which it can meet its energy requirements, resulting in decreased adipocyte size, increased adipocyte death and thus development of lipoatrophy. However, there are limitations to this theory, as both *in vitro* and *in vivo* experiments show that mitochondrial dysfunction can occur irrespective of mtDNA levels[282]. In addition, NRTIs have been shown to be associated with reductions in both mRNA expression of adiponectin, and expression of SREBP which have been more commonly associated with PI use[283].

Insulin resistance has been associated with NRTIs, which may have a role in lipoatrophy; NRTI-treated PI-naïve HIV-infected males with lipoatrophy have markedly reduced levels of adiponectin (levels of which are found to be lower in obesity, and is believed to lower insulin resistance[284]) mRNA and protein secretion[285].

Some of the potential pathways of pathogenesis between use of PIs and NRTIs and LS symptoms are summarized in Figure 1 9 (adapted from Carr (2003)[286]). The role that other classes of ART (fusion inhibitors and chemokine co-receptors antagonists), if any, play in LS in not known, reflecting their more recent introduction to clinical practice. Furthermore, any

associations with newer drugs may be complicated by their use in patients who are already highly ART experienced. Moreover, patients with LS may be switched to newer drugs which are designed to be less conducive to LS leading to confounding by indication[287-289] where erroneous associations between newer drugs and current LS may be made, rather than true associations between past drugs and current LS.

Figure 1-9: proposed pathways of protease inhibitor and nucleoside reverse transcriptase inhibitor mediated lipodystrophy syndrome



adapted from Carr 2003[286]

On the basis of extensive documentation of stavudine-associated toxicities, in 2006 the WHO highlighted the need to reduce stavudine use and to monitor associated toxicities in areas where this drug is the first option for beginning ART[290-296]. Analysis of 2190 HIV-infected adults living in Rwanda revealed significant 2-3 fold increased risk of lipoatrophy and early (<6 months) neuropathy associated with stavudine use[297]. Indeed, the most common reason for switching ART regimen was stavudine-associated toxicity in a cohort of 559 women followed up for a median of 33 months in Uganda[298], echoing a previous South African study where over one in five of ART substitutions occurred due to stavudine-associated symptomatic hyperlactataemia, lipodystrophy or peripheral neuropathy[299].

1.5 Potential sequelae of lipodystrophy syndrome

1.5.1 Cardiovascular disease

Because of increases in life expectancy due to HAART[72], there is on-going surveillance of CVD in HIV-infected individuals as they survive to ages where rates of CVD have been shown to increase in HIV-uninfected subjects[300-303]. In a retrospective analysis of a North American cohort of over 30,000 individuals, the age and gender-stratified relative risk of CVD was significantly higher in HIV-infected compared with HIV-uninfected individuals. Moreover, following adjustment for known CVD risk factors, there was a significant two-fold increased risk of CVD in patients receiving ART compared with those ART-naïve. Taken together, these findings suggest both HIV-dependant and ART-dependant pathways may have a role in the development of CVD[304].

An Italian cross-sectional study investigated differences between HIV-patients on ART and an ART-naïve group. The patients on ART had a significantly higher incidence of dyslipidemia, and a non-significant increased carotid intima media thickness (CIMT) which is correlated to extent of coronary atherosclerosis[305]. Furthermore, within the ART group the presence of LS was associated with increased CVD risk and subclinical carotid atherosclerosis[306]. In a US-based study of 253 adults, CIMT was significantly thicker in HIV-infected subjects compared to uninfected controls[307]. In a study in HIV-infected women, CIMT was significantly higher in PI-treated individuals compared to both non-PI-treated individuals and HIV-uninfected controls, indicating that the effect of PIs may be via direct endothelial damage, or indirect via other known cardiovascular risk factors[308].

In the Data Collection on Adverse Events of Anti-HIV Drugs (DAD) study involving 33,347 HIV-infected individuals across three continents, the incidence of myocardial infarction (MI) increased with longer exposure to HAART, with an adjusted relative rate per year of exposure of 1.26 (95% confidence interval, 95% CI: 1.12, 1.41)[309]. Analysis of the same study population found that the NRTIs abacavir and didanosine were associated with an increased risk of MI, even after adjusting for established CVD risk factors. Furthermore, risk of MI was reduced after cessation of treatment with these drugs[310].

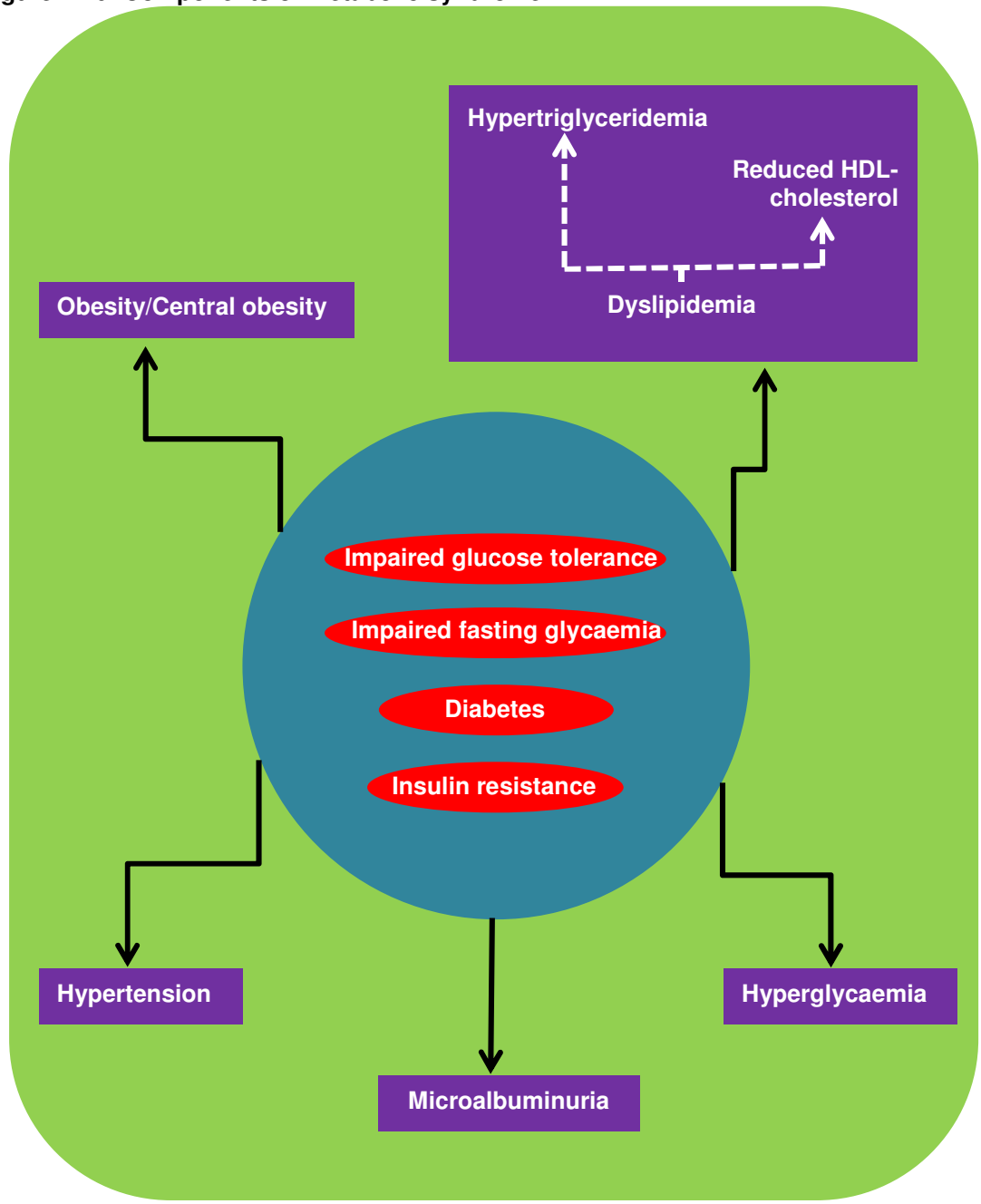
Significant positive associations between metabolic abnormalities and both progressive atherosclerosis and CVD have been reported[311], with evidence of advancing development of atherosclerotic plaques during adolescence[312]. Examination of autopsies of 204 subjects aged 2-39 years who had died from all-causes (mainly trauma) suggests that not only do young people develop the atherosclerotic plaques characteristic of CVD, but the severity of asymptomatic coronary and aortic atherosclerosis increases as the number of cardiovascular risk factors (e.g. serum triglyceride, total cholesterol, LDL-cholesterol, HDL-cholesterol etc.) increases[313]. Given that atherosclerotic plaques develop in HIV-uninfected adolescents and that

ART is associated with metabolic alterations, ART may both induce metabolic abnormality in HIV-infected children and potentially accelerate development of atherosclerosis. ART is also increasing survival in HIV-infected patients, so that many are surviving to middle-age and beyond where CVD tends to manifest clinically. With improved survival of HAART-treated HIV-infected children[117], it is conceivable that many may survive past early adulthood. Increased survival may in itself increase the risk of CVD as age is an important risk factor for CVD[314]. Moreover, the increasing survival may mean that survivors are living with an abnormal metabolic profile for longer. The risk of developing CVD may therefore be affected by both increasing age and hyperlipidemia.

Children and adolescents with high cholesterol levels are more likely to have high levels in adulthood[315], and this may also apply in the context of LS. Furthermore, cholesterol levels in HIV-infected children treated with PIs have been shown to be similar to those observed in children who are heterozygous for familial hypercholesterolemia (who usually develop CVD in middle age).[316] In a comparison of HIV-infected children with matched uninfected controls (median age 9 years), the HIV-infected group had higher levels of triglyceride and significant increased CMIT. Regression analysis showed that duration of ART predicted CMIT while traditional atherosclerosis risk factors, HIV disease factors and duration of PI use did not[317]. It is therefore possible that LS in adolescence may lead to CVD occurring at younger ages compared to the non-HIV infected population due to the cumulative lifetime effects of hyperlipidemia and insulin resistance.

The *metabolic syndrome*, which includes many of the characteristics of LS, describes a number of metabolic abnormalities that are related to increased risk of not only CVD, but also of obesity and of diabetes mellitus[318]. It is commonly defined as ≥1 of: impaired glucose tolerance, impaired fasting glycaemia, diabetes or insulin resistance, with ≥2 of: obesity, dyslipidemia, hypertension, microalbuminuria or hyperglycaemia (Figure 1-10). Although it is difficult to separate the independent effects of HIV, there is evidence that prevalence of diabetes is similar between HIV-infected ART-naïve individuals and those who are both uninfected and ART-naïve: prevalence is greater in HIV-infected ART-treated subjects in comparison to both the former groups[319,320]. Furthermore, specific ART drugs have been shown to have different effects on the extent to which insulin resistance may occur[321].

Figure 1-10: Components of metabolic syndrome



Metabolic syndrome is commonly defined as 1 of the conditions detailed in red with at least 2 of the conditions detailed in purple (see Eckel *et al* 2005, for details of different definitions and thresholds[318])

Thus the metabolic changes seen in LS, summarized in Table 1-4 (reproduced from Grunfeld *et al* [322]) are important in the development of CVD and diabetes[323]. For example, the changing ratio of increased low density lipoprotein (LDL) cholesterol to decreased high density lipoprotein (HDL) cholesterol, and increased levels of mediators of inflammation such as TNF are conducive to atherosclerotic plaque genesis[324].

Table 1-4: Summary of metabolic changes and body fat abnormalities seen in lipodystrophy syndrome (LS) and their relationship with HIV infection, and viral suppression. Reproduced from Grunfeld *et al* 2008[322]

		Effect of untreated or ineffectively treated HIV	Changes associated with viral suppression	Changes with specific antiretroviral therapy	Role of body shape changes
Lipid metabolism	**High density lipoprotein (HDL) cholesterol**	Decreases early in infection	Increases modestly but not to pre-morbid levels	Greater increases seen with NNRTI and possibly atazanavir than other PI	Increased visceral adipose tissue and upper trunk fat associated with low HDL
	Low density lipoprotein (LDL) cholesterol	Decreases later in infection	Increases modestly	-	-
	Triglycerides	Increases in late infection	Decreased in early study of zidovudine mono-therapy	No decrease or even further increase with HAART regimens containing some PIs Increases may also may be seen with stavudine and efavirenz	Increased visceral adipose tissues and upper trunk fat Lower leg fat associated with increased triglyceride levels
Glucose metabolism	**Insulin sensitivity**	Conflicting evidence of increase or decrease	Decreases insulin sensitivity independent of regimen; some insulin resistance represents restoration to health	Some PIs decrease NRTIs (particularly stavudine) may also contribute to decreases	Increased visceral adipose tissue and upper trunk fat and lower limb fat associated with insulin resistance
	Insulin secretion	No evidence of an effect	No evidence of an effect	Some PIs may decrease insulin secretion	-
	Fasting glucose	No evidence of an effect	-	Some PIs increase endogenous glucose production	-
	Glucose tolerance	No evidence of an effect	-	Higher rates	-
	Diabetes	No evidence of an effect	Possible higher prevalence of diabetes	Higher prevalence and incidence of type 2 diabetes Associated with PI (especially indinavir and ritonavir) Associated with NRTI especially stavudine	-
Body composition	**Lean body mass**	Decreases disproportionately with severe wasting	Increases modestly with initiation of effective HAART	No consistent evidence of direct drug effects	-
	Peripheral fat	Decreases proportionally with wasting	Initially increases with initiation of effective HAART	Subsequent preferential depletion of subcutaneous fat in arms, arms, legs, and buttocks more than upper trunk	-

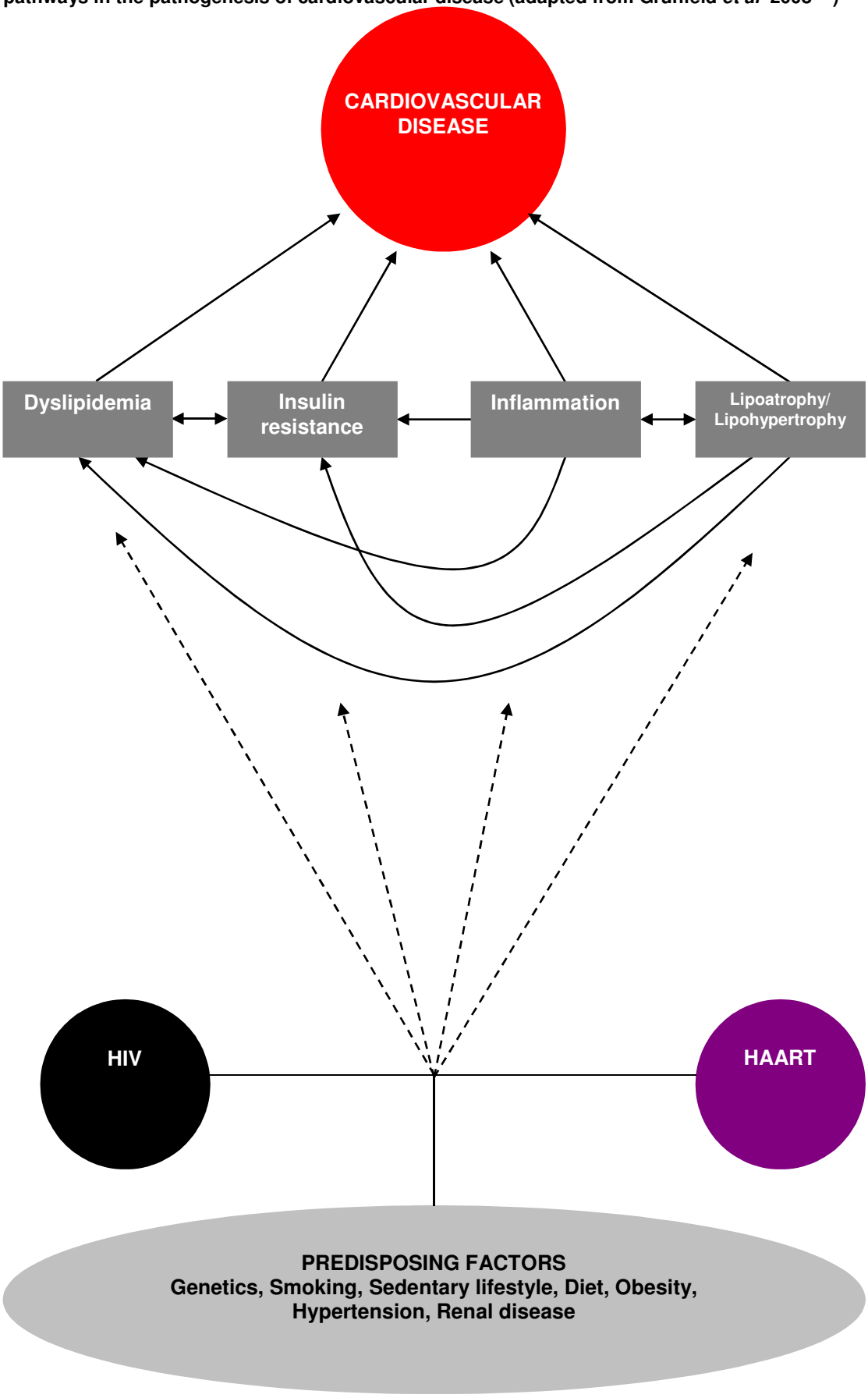
	Effect of untreated or ineffectively treated HIV	Changes associated with viral suppression	Changes with specific antiretroviral therapy	Role of body shape changes
			Associated with some NRTIs (especially thymidine analogues) Role of different PIs and NNRTIs unclear	
Visceral fat	Decreased but relatively preserved Weight cycling during episodic weight loss and regain	Increases with initiation with effective HAART	Preserved or increased visceral fat in some patients on HAART Association with specific drugs unclear	-

It is possible that HIV may serve as a marker to identify a subgroup in the general population with an increased prevalence of traditional CVD risk factors such as sedentary lifestyle, smoking hypertension etc. Alternatively the HIV infection may have an independent pathogenic role, stimulating CVD through inflammation. ART may affect the risk of developing traditional CVD risk factors or have an independent pathogenic role. However it may be that all these processes work together[325]. Figure 1-11 (adapted from Grunfeld et al [322]) highlights the possible interplay between the postulated pathways.

While there is accumulating evidence that long term ART use may lead to a metabolic profile conducive to CVD in a subset of patients, the ART-associated risk of MI in HIV-infected adults is offset by the benefits of maintaining health and prolonging life[326]. There has been an upward trend in the prevalence of being overweight[e] in children resident in the developed world in recent years[327], and thus it may be difficult to tease out the effects of ART-associated body fat disorder where HIV-infected children are being raised in an obesogenic environment[328]. Furthermore, the difficulty of assessing adiposity against the backdrop of changes in body habitus during adolescence and puberty[329] complicates the situation further. Although there is abundant evidence to support the role of obesity in the pathogenesis of CVD[330,331], there is conflicting evidence regarding the role of childhood obesity as an independent risk factor[332].

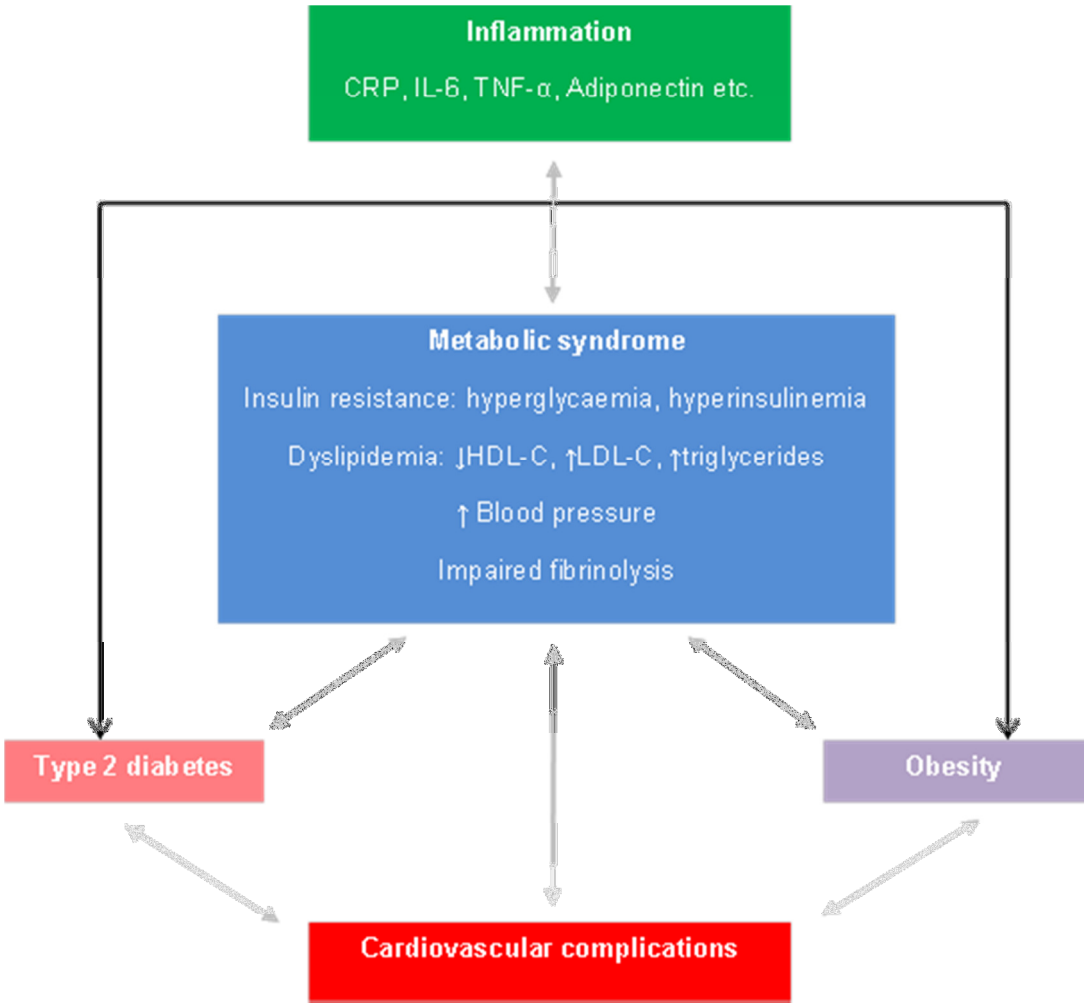
[e] Methods used to assess obesity in children include weight-for-height z-scores (with WHO and US NHANES I reference populations), >120% ideal weight for height, 85th and 95th BMI percentiles, adult BMI thresholds of BMI≥25.

Figure 1-11: postulated interplay between traditional, inflammatory and lipodystrophy-mediated pathways in the pathogenesis of cardiovascular disease (adapted from Grunfeld *et al* 2008[322])



Nevertheless, inflammation seen in obesity not only has been associated with CVD, but also other comorbidities such as diabetes (Figure 1-12). The direct effects of HIV on the inflammatory response include increases in levels of specific cytokines such as IL-6 and TNF leading to impaired cell immunity[333]. It may be possible that the risk of CVD may be influenced by inflammation induced by both HIV infection and obesity. Indeed there has been recent interest in the emergence of obesity in HIV-infected populations[334,335].

Figure 1-12: Interrelationship between metabolic abnormality and clinical outcomes (from Rana et al[336])



Double arrows represent disorders that can perpetuate and affect each other. CRP: C-reactive protein.

It has been argued that inflammation mediates insulin resistance (which itself is associated with obesity and diabetes), hypertension, prothrombosis and hyperlipidemia: inflammation and insulin have adverse effects on cardiac muscle, and the metabolic abnormalities collectively

cause cardiovascular complications[336]. A systematic inflammatory response[f] to obesity has been reported in children[337], and while obesity and LS are separate conditions, it is possible that LS will also illicit a similar inflammatory response. Furthermore, the inflammatory response to obesity/LS may be complicated by the inflammatory response to HIV. Obesity in infants has been shown to be associated with later obesity[338], and it may be that the metabolic effects and accompanying inflammatory response associated with LS in childhood may be associated with LS and the postulated inflammatory response to LS/obesity later in life.

The characteristics of a paediatric inflammatory response to LS remain unknown, as there are no cohorts of sufficient age to investigate the incidence of CVD. For example, it is unknown whether the effect of childhood LS on the inflammatory system is relatively transient, or consistent throughout adolescence in children with LS. Moreover, the independent effect of HIV on inflammation is difficult to separate, as is the effect on normal growth and development. Furthermore, as HIV-infected children may be switched to less toxic drugs, it is unknown whether the consequences of previous inflammatory damage will continue to contribute to increased risk of CVD or not, given the influence of HIV, adolescence, diet and behaviour.

1.5.2 Psychological effects

High occurrence of poor psychological profile, including mental illness, depression, feelings of alienation, and stress, has been widely reported in HIV-infected individuals[339,340], with negative implications for quality of life, markers of HIV disease and adherence to ART[341]. However, there is evidence that HAART has led to stabilization of quality of life measures over time, irrespective of slight changes in HIV-related symptoms[342]. In a cross-sectional analysis of 13,601 adolescents aged 16-18 years living in the US, self-perception of being underweight or overweight were both significantly associated with an increased risk of self-reported symptoms of depression[343]. There is a lack of research investigating the association between self-perceived body image and depression in adults who do not have underlying medical conditions. However, a meta-analysis of longitudinal studies investigating obesity (defined as BMI ≥ 30) and depression in adults reported a pooled estimate of 55% increased risk of developing depression in obese individuals over time[344].

A Brazilian cross-sectional study of HIV-infected and ART-treated adults with clinically confirmed body fat alterations (median age 42 years) indicated a range of psychological consequences of LS: of 25 patients with facial lipoatrophy, all those reporting a change in self-image lost the desire to look at themselves in the mirror, and 10 (40%) reported social disruption and isolation; furthermore, 4 (16%) reported that they would not have started ART had they known of the risk of lipoatrophy[345]. In a Canadian cross-sectional study of 77 HIV-

[f] as defined by changes in circulating acute-phase reactants and cytokines including TNF, interleukin 1 (IL-1), IL-6, interleukin-10 (IL-10), adipnectin, leptin and resistin, being correlated to comorbid conditions (insulin resistance, dyslipidemia, non-alcoholic fatty liver disease, atherosclerosis and hypercoagulation)

infected adults (median age 44 years) on HAART with body fat changes, standard quality-of-life tools were used to assess the psychological effects of lipoatrophy and lipohypertrophy. Over 70% of individuals displayed minimal depression, 23% had mild or worse depression, and 8% had moderate or severe depression, with significant association with patient lipodystrophy profile (defined as degree of self-reported fat alterations at specific body locations)[346].

Although there is increasing literature on children and adolescent pre-occupation with body image, and the relationship between negative self-image and low self-esteem[347], this is an overlooked area of research in relation to LS-associated body changes. However, it is likely that HIV-infected children will have similar anxieties about body image as other children and adolescents. This is of concern since HIV-infected children have been shown to be shorter and lighter than uninfected children of the same age[348]. The psychological impact of poor body image in HIV-infected children may be similar to that seen in other chronically ill children where either illness or treatment has affected body shape. A systematic review of body image amongst children and adolescents with cancer found that there was an association between compromised body image and levels of anxiety, depression and behavioural problems[349]. Furthermore, survivors of adolescent cancer have reported negative self-assessments of their body image compared to independent assessments, suggesting that the psychological effects of treatment or disease-related body changes persist long after treatment cessation or recovery [350].

Psychological state and adherence

Associations between low self-esteem and also feelings of shame with body changes in ART-treated HIV-infected adults have been reported[351]. This may have implications for ART adherence. In an American investigation into self-reported adherence to HAART in 1671 women, participants reported that they were more likely to stop taking HAART with self-perceived fat gain in the abdomen, arms and legs, or fat loss in the chest or arms and legs[352]. Follow up of 277 HIV-infected adults who were initially adherent to HAART found one third to be non-adherent at 20 months: the number of self-reported lipodystrophy-related symptoms was significantly associated with failure to maintain adherence after adjusting for confounding variables[353]. Many factors influence the psychological state of mind of an HIV-infected child and teenager, including the effects of living with a chronic illness, fear of stigmatization, cognitive development problems etc., adding to the complexity of pressures of adolescence. Fat alterations may also contribute additional stress. Children and particularly teenagers may associate their ART simply with the body dysmorphic adverse effects rather than the long-term health-promoting properties, leading to low adherence, viral rebound and the potential for the emergence of resistance. LS, together with other adverse effects of medication, and the fear of stigmatization and alienation have been identified as reasons for non-adherence[133,354]. Thus it is feasible that in a population where adherence may be problematic in some subjects, the

presence (or fear of) changes in body shape may cause a proportion of individuals to move from being adherent to non-adherent.

1.5.3 Life-long use of antiretroviral therapy in children

It is possible that the global incidence of paediatric LS may increase as more children have access to ART. However, the long-term survival in HIV-infected children in terms of co-morbidities is yet to be investigated fully since there are few longitudinal studies in children which have accumulated sufficient follow-up time and data. Investigations in adults regarding emergence of factors such as CVD, and diabetes in ART-treated HIV-infected adults cannot necessarily to be extrapolated to similar populations of children because of concurrent adolescent physiological development, the different pharmokinetic properties of ART drugs in children compared to adults, and furthermore, different dosing in adults and children. The roles of early life growth, nutrition and events[355,356] in otherwise healthy children have been associated with later cardiovascular risk, and guidelines aimed at promoting health in children in relation to heart disease are being produced[357]. This may indicate the need to investigate the possible concurrent effects of HIV infection and ART[357] . Moreover, knowledge of LS in children, and its applicability to wider populations of HIV-infected children and adolescents, is limited by previous research having been restricted to cross-sectional design, small size, single-centre construction, and highly specific outcomes (Table 1-5). However, as survival rates in ART-treated HIV-infected children are increasing, and with the availability of data from aging paediatric cohorts, research in this area is now feasible. Thus it has become possible to investigate the implications of longer-term use of ART, including at times of adolescent development, on LS. Furthermore, the trends in ART use in HIV-infected children can be explored, i.e. cumulative exposure, switching between regimens, and adverse effects in addition to LS. Moreover, the emergence of CVD risk factors in ART treated children, or in adults who were treated with ART when children, can be examined.

Table 1-5: Summary of published studies looking at lipodystrophy in children and adolescents

Study	Design	Location	Number of participants	Age range (years)	Main outcomes	
					Metabolic	***Fat alterations***
Amaya *et al* (2002)[195]	Cross-sectional	USA (single-centre)	40	2-16	Hyperlipidemia	Lipoatrophy and/or lipohypertrophy
Arpadi *et al* (2001)[200]	Prospective 52-week follow-up	USA (single-centre)	28	4-12	-	Lipoatrophy and/or lipohypertrophy
Aurpubil *et al* (2007)[201]	Prospective 144-week follow-up	Thailand (single-centre)	90	7±2.9*	Hypertriglyceridemia, hypercholesterolemia	Lipoatrophy and/or lipohypertrophy
Beregszaaszi *et al* (2005)[211]	Prospective 104-week follow-up	France (multicentre)	130	10.0 (9.4, 10.7)**	Hypertriglyceridemia, hypercholesterolemia, hyperinsulinaemia[#]	Central/peripheral skinfold thickness[†]
Bitnun *et al* (2003)[212]	Cross-sectional	Canada (single-centre)	50	3-18	Serum cholesterol, serum triglyceride, serum insulin	Visceral to subcutaneous adipose tissue ratio
Bockhorst *et al* (2003)[202]	Cross-sectional	USA (single-centre)	26	4-15	Serum cholesterol, Serum triglyceride, Serum insulin	Lipoatrophy and/or lipohypertrophy
Carter *et al* (2006)[203]	Retrospective 7-year follow-up	USA (multicentre)	178	0-15	Hypercholesterolemia, hypertriglyceridemia	Lipoatrophy and/or lipohypertrophy
Cheseaux *et al* (2002)[316]	Cross-sectional	Switzerland (multicentre)	66	<17***	Plasma cholesterol, plasma triglyceride	-
Desai *et al* (2008)[204]	Prospective 72-week follow-up	USA (single-centre)	48	6-15	Hyperlipidemia	Lipoatrophy and/or lipohypertrophy
Dos Reis *et al* (2011)[358]	Cross-sectional	Brazil (single-centre)	119	6-19	Serum cholesterol, Serum triglyceride	-
Ene *et al* (2007)[205]	Cross-sectional	Belgium (single-centre)	88	3-19	Hyperlipidemia[##]	Lipoatrophy and/or lipohypertrophy
European	Cross-sectional	Europe	477	3-18	Serum cholesterol,	Lipoatrophy,

Paediatric Lipodystrophy Group (2004)[209]		(multicentre)			serum triglyceride	lipohypertrophy
Farley *et al* (2005)[196]	Cross-sectional	USA (multicentre)	1812	4-19	Hypercholesterolemia	-
Jaquet *et al* (2000)[194]	Cross-sectional	France (single-centre)	39	9.1±4.0*	Serum cholesterol, serum triglyceride, serum insulin	Lipoatrophy, lipohypertrophy
Kim *et al* (2009)[359]	Retrospective 10-year follow-up	USA (single-centre)	178	-	Hypercholesterolemia	-
Lainka *et al* (2002)[360]	Cross-sectional	Germany (single-centre)	37	1-17	Serum cholesterol, serum triglyceride, serum insulin	-
Melvin *et al* (2000)[213]	Cross-sectional	USA (single-centre)	23	4-18	Serum cholesterol, serum triglyceride, serum insulin	Percentage body fat by location
Parakh *et al* (2009)[206]	Cross-sectional	India (single-centre)	52	2-12	Hypercholesterolemia, hypertriglyceridemia	Lipoatrophy and/or lipohypertrophy
Resino *et al* (2010)[210]	Retrospective 192-week follow-up	Spain (single-centre)	27	4-13	-	Lipoatrophy, lipohypertrophy
Rhoads *et al* (2011)[361]	Prospective cohort	United Kingdom (single-centre)	449	6.6 (3.3, 9.9)**	Hypercholesterolemia	
Sanchez Torres *et al* (2005)[207]	Cross-sectional	Spain (single-centre††)	56	1-18	Hypercholesterolemia, hypertriglyceridemia	Lipoatrophy and/or lipohypertrophy
Solorzano Santos *et al* (2006)[208]	Prospective 24-week follow-up	Mexico (single-centre)	24	0-15	Hypercholesterolemia, hypertriglyceridemia	Lipoatrophy and/or lipohypertrophy
Tassiopoulos *et al* (2008)[197]	Prospective 200-week follow-up	USA (multicentre)	2122	0-22	Hypercholesterolemia	-

***mean ± standard error, **median (interquartile range), ***range not given, #age and gender define thresholds to define cases, ## >95th centile of age and gender defined thresholds, †case defined as >3 standard deviations away from the mean. ††not explicitly state**

1.6 Key points

- While the global incidence of new HIV infections among children has stabilized over the last decade, the prevalence of children living with the infection has increased to an estimated 2 million in 2007.

- The increased number of children living with HIV is attributable to improved access to ART with global coverage of 28% in 2009. As global efforts move towards universal access, more children are likely to receive treatment.

- LS is defined by alterations in body fat, with lipohypertrophy and/or lipoatrophy in specific locations, with/without distinctive metabolic abnormality (at least one of: hypercholesterolemia, fasting hypertriglyceridemia, and glucose intolerance). Thus LS can manifest as a heterogeneous phenotype.

- PIs, NRTIs and NNRTIs have been associated with increased risk of LS in a large number of adult and paediatric studies. Although a number of pathological mechanisms have been hypothesized, the relationship between metabolic abnormality and body fat alterations is still uncertain.

- There is evidence to suggest that lipodystrophy may be conducive to the development of CVD: HIV-infected individuals are both surviving beyond middle age when clinical outcomes associated with CVD traditionally occur, and also have a metabolic profile consistent with early development of CVD.

- Changes in body shape due to lipoatrophy and lipohypertrophy may lead to a negative self-image and may have implications for adherence to ART.

- Because treatment is life-long, HIV-infected children are likely to accumulate long durations of ART and exposures to multiple regimens: the impact of this on the development of LS is unknown.

- LS in children and adolescents is an increasingly important issue due to the possible effects on cardiovascular and psychological health.

- While there have been a number of paediatric studies investigating lipodystrophy, many of their results have been limited by being cross-sectional in nature, or of small size and limited number of sites.

2. Rationale, objectives and data

2.1 Rationale for this research

While there has been a wealth of research into LS in HIV-infected adults in the past decade (Appendix H: Table H-1), there have been fewer paediatric studies (Table 1-5), despite LS being a key area of concern for HIV care in children. Studies to date have indicated that up to one-third of HIV-positive children receiving treatment have LS[194,195,209], highlighting its importance as an area of study. Furthermore, the scale-up of ART in resource-limited settings will result in increasing numbers of children with HIV using treatment, which should be life-long once started. LS not only has the potential to decrease quality of life and treatment adherence among HIV-infected children and adolescents, but may also negatively impact on their future cardiovascular health.

Most paediatric studies to date have been restricted by their cross-sectional nature, single-centre design, and small number of study participants (Table 1-5). Although there is evidence that LS manifestation and risk factors are similar in children and adults, specific paediatric research is required, as results from adult studies cannot be generalised to children. Moreover, previous studies in children have tended to investigate either body fat alterations or metabolic abnormality in isolation, with few examining both. There have been few prospective paediatric studies[196,202,203,208], and thus relatively little is known about the emergence of LS in the context of pubertal changes and childhood growth. Prospective data are urgently needed to better understand factors associated with the emergence, persistence and progression/regression of body fat changes and metabolic abnormalities, and to develop effective strategies to reduce or prevent, and to manage, lipodystrophy.

This thesis uses data from an on-going large prospective multicentre study of children and adolescents living with HIV where specific information on both body fat alterations and metabolic abnormality has been collected longitudinally. The large study sample provided power to perform statistical modelling to identify postulated risk factors, while prospective follow-up of children with and without LS allowed estimation of incidence of LS, investigation of the progression or regression of LS symptoms over time, and examination of the temporal relationship between changes in body fat habitus and metabolic measures. Assessment of current management strategies will be important to inform future policy and guidelines for clinical management.

2.2 PhD aim and objectives

This thesis aims to investigate paediatric lipodystrophy syndrome through active prospective surveillance of a cohort of HIV infected children and adolescents living in Europe: it will describe symptoms, identify risk factors, examine the emergence of the syndrome, and explore the relationship between the body fat alterations and metabolic abnormality.

(1) To estimate the point prevalence and incidence of LS in a population of HIV-infected children and adolescents living in Europe and to describe the phenotypes of LS in HIV-infected children and adolescents.

(2) To evaluate risk factors for the emergence of body fat alterations and metabolic abnormalities presenting in these children and adolescents.

(3) To assess prospectively the progression/regression of body fat alteration symptoms in lipodystrophy cases and associated risk factors, and to further define the relationship between body fat alterations and metabolic abnormality.

(4) To model longitudinal changes on markers of lipid and carbohydrate metabolism to clarify the cumulative effect of HAART exposure over time on dyslipidemia

(5) To report the clinical management of HIV-infected children and adolescents with LS with respect to interventions aimed at improving lipodystrophy symptoms.

2.3 Outline of thesis

Chapter 2 describes the structure of the cohort with respect to data sources, methodology of data collection, and description of variables, key outcomes, and statistical methodology. In Chapter 3, the subjects are described based on characteristics at the time of recruitment; this includes background information such as ethnicity and age, but also characteristics related to HIV, i.e. mode of acquisition, clinical status, degree of immunosuppression etc. Chapter 3 also details estimates of prevalence of LS. Several estimates of prevalence are made, reflecting the different metabolic abnormality and body fat alteration symptoms reported in the published literature. Furthermore, differences in the concentration of metabolites, and anthropometric measures between subjects with LS and those without are explored in this chapter.

Risk factors for LS, including fat alteration and metabolic abnormality, in cross-sectional analyses at recruitment are reported in chapters 4 and 5. These analyses will allow ascertainment as to whether risk factors are different for body fat alterations and metabolic abnormality. The role of ART as a possible risk factor is examined by investigating individual drugs, categories of ART, and specific regimens.

The focus of chapter 6 is the emergence of LS, with estimates of incidence presented and associated risk factors identified: this allows determination as to whether risk factors at recruitment are similar to those associated with emergence. In addition, a comparison between subjects with regression of LS symptoms, and those with progression is conducted.

The relationship between fat alteration and metabolic abnormality is investigated in Chapter 7: including analyses investigating temporal relationships between body fat alterations and metabolic abnormality. Multi-level modelling is used to examine the relationship between LS and concentrations of cholesterol and fasting triglyceride. In this chapter the management of LS within the cohort is also described. Chapter 8 includes a full discussion of the results, with implications for future work, and strengths and weakness of the study.

2.4 Key outcomes

The definition of LS used in this study was the occurrence of any body fat alterations and/or any metabolic abnormality, regardless of severity. Box 2-1 and Box 2-2 give the definitions of body fat alterations and metabolic abnormality respectively.

Box 2-1: Definition of fat alterations

One or more of the following:

- Facial lipoatrophy: sunken cheeks, sunken eyes, or prominent zygomatic arch
- Arm lipoatrophy: skinny arms, prominent veins, prominent muscularity, or prominent bones
- Leg lipoatrophy: skinny arms, prominent veins, prominent muscularity, or prominent bones
- Abdominal lipohypertrophy: increased abdominal girth
- Dorso-cervical lipohypertrophy: fat accumulation in the neck/back ("buffalo hump")
- Breast lipohypertrophy: breast enlargement

Specific manifestations of body fat alterations were considered as outcomes for different analyses throughout this study:

- Any body fat alterations
- Lipoatrophy only (no lipohypertrophy)
- Lipohypertrophy only (no lipoatrophy)
- Combined lipoatrophy and lipohypertrophy occurring together

Both hypercholesterolemia and hypertriglyceridemia were diagnosed according to gender and age-defined thresholds (Appendix B: Table B-1 and Table B-2).

Box 2-2: Definition of metabolic abnormality

One or more of the following:

- Hypercholesterolemia (assessed according to age and gender thresholds)
- Fasting hypertriglyceridemia (assessed according to age and gender thresholds)
- Impaired glucose tolerance

Specific manifestations of metabolic abnormality were considered as outcome variables for various statistical models throughout this investigation:

- Any metabolic abnormality
- Hypercholesterolemia only (no fasting hypertriglyceridemia)
- Fasting hypertriglyceridemia only (no hypercholesterolemia)
- Concurrent hypercholesterolemia and fasting hypertriglyceridemia

Outcomes including impaired glucose tolerance were not included in analyses because of small numbers of study subjects who had this condition.

2.5 Data source

In 2004 the European Paediatric Lipodystrophy Group investigated body fat alterations and metabolic abnormality in a cross-sectional analysis of nearly 500 children recruited from paediatric HIV centres participating in the ECS and the Italian Register of HIV Infection in Children[209]. This study formed the basis for a larger investigation designed to examine body fat abnormalities and metabolic abnormality using active surveillance through the establishment of the European Paediatric Lipodystrophy Cohort.

2.5.1 The European Paediatric HIV and Lipodystrophy Cohort

Recruitment of subjects into the cohort occurred between 2007 and 2009. Participants were enrolled from a selection of participating paediatric HIV centres within the ECS in HIV-infected Pregnant Women and their Children, and the Italian Register of HIV Infection in Children. The ECS is a multi-centre, multi-country cohort comprising of HIV infected pregnant women and their children which was first set up in 1986[168]. The Italian Register for HIV infection in children was established in 1985 to establish the extent of paediatric HIV infection in children and explore its epidemiology across the country[362].

To be eligible for recruitment in this study, patients must have been HIV-infected and aged less than 18 years. Subjects were excluded from recruitment if they were aged less than 2 years old or had been treated with anabolizing steroids in the previous six months. Recruitment took place in three European countries: Belgium, Italy and Poland. Within each country, subjects were recruited from University teaching hospitals with specialist paediatric HIV services. The participating paediatric centres are presented in Box 2-3 and Figure 2-1, Figure 2-2 and Figure 2-3

Box 2-3: Paediatric HIV centres participating in the European Paediatric Lipodystrophy Cohort.

Belgium

Hospital Universitaire St. Pierre, Brussels*

Hospital Universitaire St. Luc, Brussels*

Centre Hospitalier Regional La Citadelle, Liege*

Italy

Pediatrics Clinic, Brescia University, Brescia†

Departments of Pediatrics, University of Florence, Florence†

San Martino Hospital/University of Genoa, Genoa*†

San Paolo Hospital, Milan†

Luigi Sacco Hospital/University of Milan, Milan*†

University Federico II, Naples†

Padua University, Padua*†

Bambino Gesù Hospital, Rome†

University of Turin, Turin†

Poland

Medical University of Warsaw/Regional Hospital of Infectious Disease, Warsaw *

*** Member of the European Collaborative Study on HIV-infected Women and their Children**
† Member of the Italian Register for HIV Infection in Children

Children and adolescents receiving treatment from these centres were invited to participate in the study between January 2007 and December 2008. The clinician responsible for the long-term treatment of the child would approach the child and family and introduce them to the study aims and protocol. Patients and their families were free to refuse to participate. Parents and guardians were involved in the enrolment process. Informed consent was sought from each participant according to the research ethics approval procedures at each site.

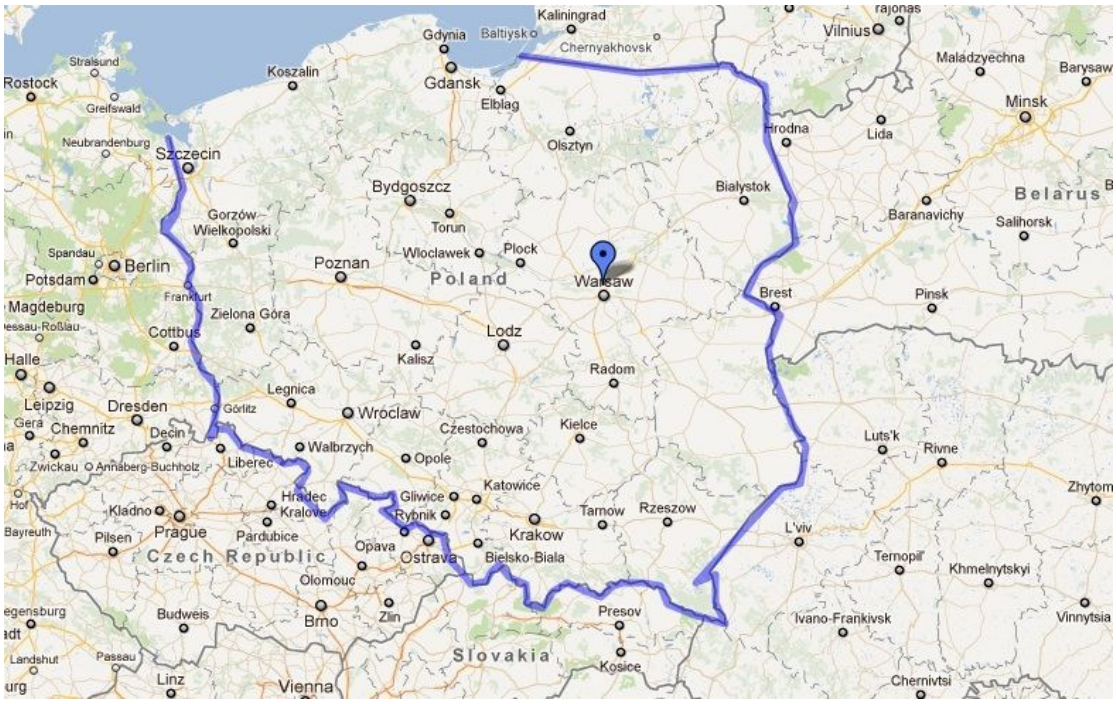
Figure 2-1: Clinical sites located in Belgium

Hospitals: Hospital Universitaire St Pierre, Brussels; Hospital Universitaire St Luc, Brussels; Centre Hospitalier Regional La Citadelle, Liege

Figure 2-2: Clinical sites located in Italy

Hospitals: Brescia University, Brescia; University of Florence, Florence; San Martino Hospital, Genoa; San Paolo Hospital, Milan; Luigi Sacco Hospital, Milan; University Frederico II, Naples; Padua University, Padua. Bambino Gesu Hospital, Rome; University of Turin, Turin

Figure 2-3: Clinical site located in Poland



Hospital: Medical University of Warsaw, Warsaw

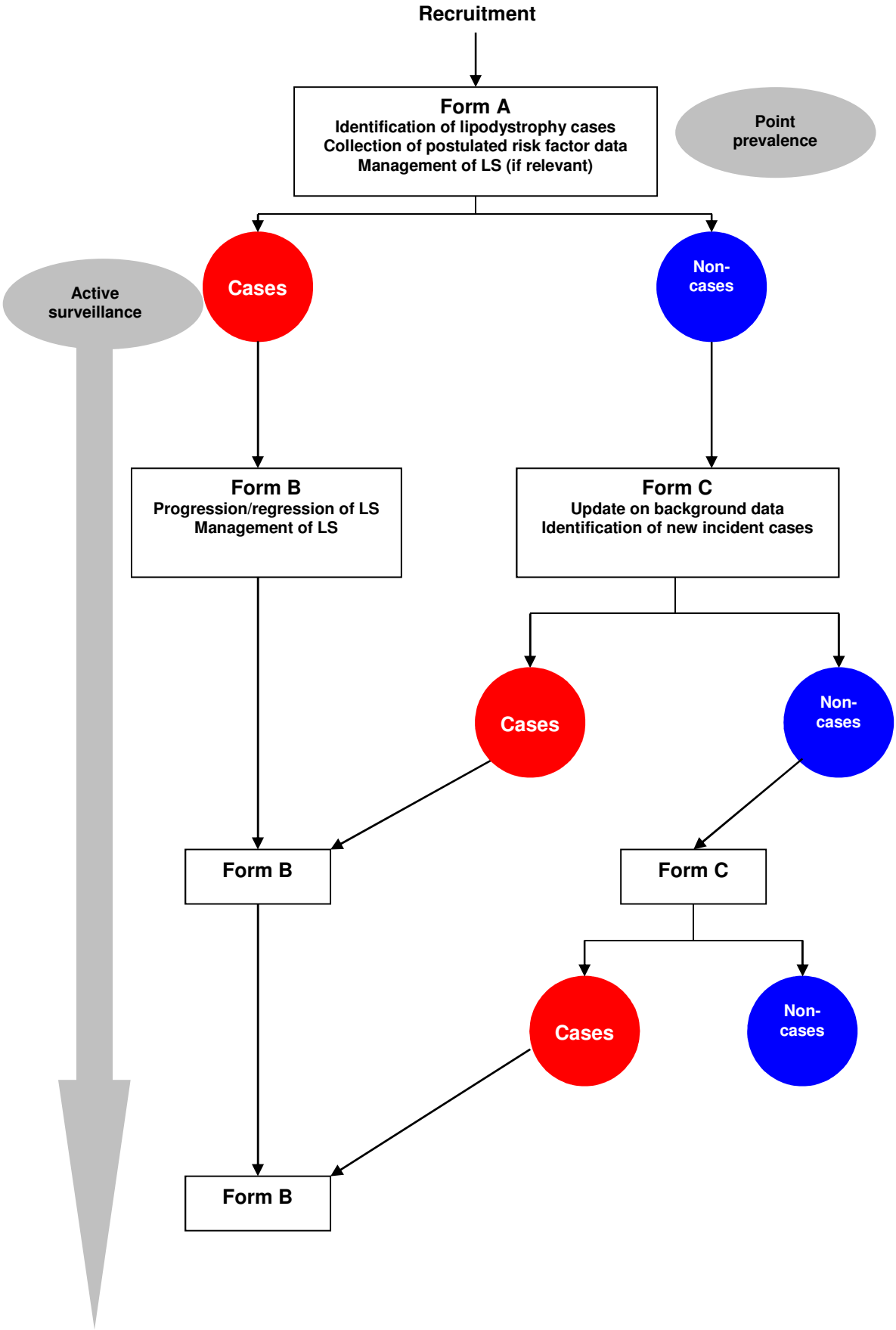
2.6 Data collection

The study is a prospective observational cohort monitoring L by active surveillance. Figure 2-4 illustrates the study design.

Data were collected on study-specific paper data forms: all forms were in English language, and all data were collected in English. At enrolment, clinicians completed a recruitment questionnaire (Form A) for each subject. At this point complete information on socio-demographic factors and ART history was collected, as well as metabolic and fat disorder data to categorize cases (of LS) or non-cases. At subsequent follow-up stages, Form B and Form C were completed on behalf of subjects: both forms were designed to collect data updating information on socio-demographic and ART regimen. Form B was devised to specifically collect information updating LS-related symptoms, whilst Form C was constructed to capture new incident cases.

Data collection forms A, B and C are reproduced in Appendix B.

Figure 2-4: Flow chart summarizing data collection in the prospective cohort



2.6.1 Specific data items collected and key variables created

Data collection forms were completed, and relevant clinical assessments conducted by the child's long-term physician at scheduled visits to the paediatric HIV clinic. Although these visits were part of the subject's on-going care, a visit where a data collection form was completed is referred to as a "study visit" in this thesis.

Data collected in Form A

Form A (completed at recruitment) consisted of sections on socio-demographics (including date of birth, ethnicity), birth details (including birth weight and gestational age), HIV-related variables (including mode of HIV acquisition, current and maximum CDC clinical status[363], current and nadir CD4 count, current CD4%, HIV RNA viral load), ART (complete history and current use), HCV co-infection, pubertal stage, anthropometry, clinically-assessed body fat alterations and laboratory tests (Appendix B) .

Box 2-4 summarizes CDC-defined clinical status of HIV used in this study: specific definition of these CDC-categories is provided in Appendix B.

Box 2-4: Definition of CDC-defined HIV clinical status

N: No symptoms

A: Mild symptoms

B: Moderate symptoms

C: Severe symptoms

For specific details of symptoms see Section A2.1 in Appendix 2

CD4 count variables were classified as degree of immunosuppression with reference to published age-based thresholds from the CDC (Appendix B: Table B-3) [34,35]: Box 2-5

Box 2-5: Definition of CDC-defined immunosuppression

Stage 1: No immunosuppression

Stage 2: Moderate immunosuppression

Stage 3: Severe immunosuppression

For specific details of symptoms see Table B-3 in Appendix B

Viral load measurements were used to create a detectable viral load variable, with undetectable viral load defined as <50 copies/ml.

Complete ART history was collected, with drug names, dates of start and stop (if applicable), together with current use. For each drug entered on form A, two dichotomous variables were created: ever-use and current use of the drug. A variable was created for the use of HAART at recruitment: a conservative definition was used: use of ≥1 NRTI concurrently with either ≥1 NNRTI or ≥1 PI. Triple class therapy was defined as ≥1 NRTI with ≥1 NNRTI and ≥1 PI. NRTI mono-therapy was defined as use of NRTI drugs, but no NNRTI nor PI. Total duration of ART use was estimated using the interval between first exposure to ART and date of recruitment (or date of complete cessation of ART use, if this had occurred).

Specific anthropometric measures recorded at recruitment were weight (kg), height (cm), waist circumference (cm), and hip circumference (cm). A variable for body mass index (BMI) was created as follows:

$$BMI = {weight\ in\ kg}\Big/{(height\ in\ m)^2}$$

A variable for waist/hip ratio (both in cm) was also created. Consistency checks were also performed on these two new variables.

Tanner score for puberty[364] was recorded as a categorical variable (I – V). Collected Tanner score was validated against age. A new categorical variable for puberty was created with the following levels: (i) Tanner I (pre-pubescent children), (ii) Tanner II-IV (children and adolescents who were undergoing puberty), and (iii) Tanner V (adolescents who had completed puberty).

Body fat alterations

Body fat alteration was collected in an ordinal variable with four levels: no alterations, mild symptoms, moderate symptoms and severe symptoms. Mild symptoms were defined as those only noticeable when specifically inspected, moderate symptoms as readily obvious to the child or carer, and severe symptoms as those obvious to the casual observer. The paediatrician completing the form was asked to record such assessments regarding fat loss in the face, arms, legs and buttocks, and fat accumulation in the trunk, neck, and breast, according to the criteria outlined in Box 1-1.

Assessments of body fat alterations were made according to guidance included in the study protocol and by a clinician familiar with the patient through their duration of providing care. Details of fat alterations at each of the body locations illustrated in Box 1-1 were collected.

Metabolic Profile

Test results for the following were recorded: total cholesterol (mg/dL), HDL-cholesterol (mg/dL), LDL-cholesterol (mg/dL), triglycerides (mg/dL), glucose (mg/dL), and insulin (mcU/ml), together with the sample date and whether sampling occurred in the fasting or non-fasting state. A variable for non-HDL cholesterol was created as the difference between total cholesterol and HDL-cholesterol.

Dyslipidemia and glucose disorder

Two tables were included in Form A, for use by the clinician in determining whether the child had hypercholesterolemia and/or hypertriglyceridemia based on most recent measurements. These thresholds were defined according to gender and age (Appendix B: Table B-1 and Table B-2). Impaired glucose tolerance was defined as either impaired fasting glucose 110-125mg/dL or impaired glucose tolerance (fasting glucose < 126mg/dL and glucose value 2 hours post oral glucose tolerance test of 140-199mg/dL).

Management of lipodystrophy syndrome

For children with LS, the data collections forms contained a section on the management of LS, including the use of medications (e.g. growth hormone, lipid lowering agents, steroids, or insulin-sensitizing agents), specific dietary interventions, increased physical activity, and surgery.

Data collection form B

Data Form B (Appendix B) was designed to collect information on subjects who had been categorized as having LS (by the treating paediatrician) at the previous data collection round.

Variables that were collected and updated as detailed for Form A included:

- Current CDC-defined clinical condition
- CD4 count and percentage
- HIV-RNA viral load
- Anthropometric measurements and pubertal stage
- Clinical assessment of body fat alterations by body location
- Metabolic profile
- ART: *change* in treatment, with reasons for stopping previous regimen (classified as treatment failure, body fat alterations, dyslipidemia, some combination of the three, other)

Data collection Form C

Data collection Form C (reproduced in Appendix B) was designed to collect follow-up data in subjects who had not previously been categorized as having LS.

Variables that were collected and updated as detailed for Forms A and B included:

- Current CDC-defined clinical condition
- CD4 count and percentage
- HIV-RNA viral load
- Anthropometric measurements and pubertal stage
- Clinical assessment of body fat alterations by body location
- Metabolic profile
- Changes in ART as in Form B
- Management of LS as in Forms A and B, if applicable

Revision of data collection forms B and C

During the follow-up period, forms B and C were modified in order to collect additional data. The new data items included:

- Date of HIV diagnosis
- Age at first menarche in female subjects

Revised versions of data collection forms B and C are included in Appendix B.

2.7 Data management

The databases were centrally managed at the UCL Institute of Child Health (ICH). At each round of data collection, the Belgian and Polish paper forms were entered into a Microsoft Access (version 2010) database centrally, whereas Italian forms were entered into a database locally which was then returned to ICH. At this point the datasets were imported into STATA (version 11): all data pre-processing and analysis was conducted in STATA unless otherwise stated. The Belgian/Polish and Italian datasets were then merged. Variables such as clinical site, date of birth, country of birth, and ART start and stop dates were used to identify duplicate cases which were then excluded. At recruitment, each participant was assigned a unique identifier.

This process was conducted at recruitment, and for <3 follow-up datasets. Each cross-sectional dataset was then linked to the others by the unique identifier. Time-updated variables were created (see Section 2.8.8). Additional longitudinal variables including those detailing the regression and progression of symptoms of LS, and incidence or complete reversal of LS were created. A dataset suitable for fitting Cox proportional hazard and multilevel models was then created.

2.8 Overview of statistical methods

The statistical significance in all analyses, except where explicitly stated, was $p < 0.05$. The Wilcoxon-Mann-Whitney and the Kruskal-Wallis tests were used to assess the equality of two or more means or medians from independent samples respectively. Equality of proportions was tested using the chi- squared (χ^2) test. The Signed-rank test was used to investigate the equality of medians in paired samples[365].

2.8.1 Locally-weighted smoothing

Locally weighted smoothing and fractional polynomial modelling was conducted to explore the relationship between age at recruitment and serum lipid concentrations.

Locally-weighted scatterplot smoothing creates smooth values for a continuous outcome variable (e.g. total cholesterol) obtained by performing a non-parametric local regression with moving-width windows at the explanatory variable (e.g. age at recruitment). The regression is then weighted using a kernel approach so that the central observation in the moving window is weighted highest. The regression function is then used to predict the smoothed values for the outcome variable. The number of regressions depends on the windows' size: larger windows generate smoother (possibly biased) scatterplots; smaller windows yield rougher (though more faithful to the data) estimates with larger scatterplot variance. There are many ways to reach a compromise in this bias/variance trade off; in the analyses included in this thesis, the local neighbourhood was defined as 80% for each smoothed curve. Smoothed values for the outcome variable are thus calculated for each observed values of the variable. The standard deviation of the smoothed predictions are then calculated by taking the square root of the residual difference between the smoothed values and the observed values of the outcome variables[366,367].

Because the smoothed curve is based on a number of local regressions, the curve can be influenced to deviate several times due to the local distribution of the data, rather than being changed overall by extreme values. No assumptions are made about the functional form of the expected values of the outcome variable given the explanatory variable, and thus the model is completely data-driven.

2.8.2 Fractional polynomial modelling

These models differ from other polynomial models in that the explanatory variable can be raised to non-integer values[368] in an attempt to transform the outcome variable to linearity as well as stabilising variance and achieving residuals' normality. These models can represent a large variety of shapes using fewer terms than traditional polynomial regression models [369,370].

For each pair of variables (explanatory and outcome variable) several fractional polynomial models were constructed. Within each model, the regression was weighted using the Epanechnikov kernel function to estimate the conditional expectation of the outcome. Furthermore, models of order 1, 2, and 3 were constructed for each outcome i.e. models to the order of 1, 2 and 3 of the Box-Tidwell transformations[370] constructing the fractional polynomial. The selection of the final fractional polynomial model was then conducted by examination of gain in deviance and visual examination of the selected curves for the three orders: the model was selected on the basis of having the lowest deviance and/or the fit to data points and size of the 95% confidence intervals. Within this investigation all final models were of order two, i.e. with two components defined by the Box-Tidwell transformation (Chapter 3).

2.8.3 Logistic regression modelling

Logistic regression models[365,371] were fitted to determine the relationship between explanatory variables (e.g. age, use of PI etc.), and a dichotomous outcome variable (e.g. LS, hypercholesterolemia etc.). Explanatory variables used in analyses investigating risk factors for LS are summarized in Table 2-1, while key outcome variables are detailed in the specific methods sections in Chapters 4 and 5.

ART was examined as both individual drug and classes of ART, assessed as binary variables: current-use vs. non-current use, and ever-use vs. never-use. Subjects who were ART-naïve were categorized as non-current or never-use.

Table 2-1: **Explanatory variables used to investigate lipodystrophy syndrome**

	Variable	Coding
Demographic factors	Age at recruitment	Continuous: per year
	Sex	Binary: male/female
	Ethnicity	Categorical: white/black/other
Infection factors	CDC immune stage at recruitment	Categorical: no immunosuppression/moderate immunosuppression/severe immunosuppression
	Nadir CDC immune stage	Categorical: no immunosuppression/moderate immunosuppression/severe immunosuppression
	CDC clinical stage at recruitment	Categorical: no symptoms/ moderate symptoms/severe symptoms
	Maximum CDC clinical stage	Categorical: no symptoms/ moderate symptoms/severe symptoms
	Detectable viral load (HIV-RNA>50)	Binary: HIV-RNA≤50 copies ml^{-1}/ HIV-RNA>50 copies ml^{-1}
	Mode of acquisition	Categorical: Blood products/vertical transmission/ other
	Duration of antiretroviral therapy use at recruitment	Continuous: per year
Other factors	Hepatitis C co-infection	Binary: not co-infected/ co-infected
	Puberty (by Tanner score)	Categorical: Pre-pubertal (I)/ undergoing puberty (II-IV)/ post puberty (V)
	Body mass index	Continuous: per kg/m^2
Categories of antiretroviral therapy	Nucleoside reverse transcriptase inhibitors	Binary: no use/use
	Non-nucleoside reverse transcriptase inhibitors	Binary: no use/use
	Protease inhibitors	Binary: no use/use
Specific antiretroviral drugs	Abacavir	Binary: no use/use
	Didanosine	Binary: no use/use
	Emtricitabine	Binary: no use/use
	Kivexa	Binary: no use/use
	Lamivudine	Binary: no use/use
	Stavudine	Binary: no use/use
	Tenofovir	Binary: no use/use
	Trizivir	Binary: no use/use
	Zalcitabine	Binary: no use/use
	Zidovudine	Binary: no use/use
	Atazanavir	Binary: no use/use
	Fosamprenavir	Binary: no use/use
	Indinavir	Binary: no use/use
	Nelfinavir	Binary: no use/use
	Ritonavir booster	Binary: no use/use
	Saquinavir	Binary: no use/use
	Tipranavir	Binary: no use/use
	Efavirenz	Binary: no use/use
	Nevirapine	Binary: no use/use
Type of antiretroviral therapy		Categorical: NRTI-mono-therapy/PI-based HAART/NNRTI-based HAART/triple class therapy

Since univariable models can only investigate single risk factors in isolation, without taking into account the risk that may be due to other co-occurring factors, both univariable and multivariable models were constructed during this investigation. Multivariable models were either intuitively adjusted for specific variables, or were adjusted by variables selected by stepwise selection (the final multivariable model being selected using a backward stepwise approach). All multivariable models retained both age and duration of ART use at recruitment, as potential confounders *a priori*[234]. Furthermore, all multivariable models' intercepts contained a random effect for clinical site in order to address systematic, unobserved differences in population and treatment at each hospital. The normality assumption of the random effect in each model was checked by examination of their histograms.

Variables were systematically removed from the model containing all explanatory variables considered, until those remaining were statistically significant at the 5% level. Discarded variables were then methodically reintroduced to investigate any influence on effect size and significance. All possible variable combinations were investigated in nested models: reintroduced variables were retained and the process was repeated if the reintroduction resulted in a change in effect size >10% in other variables, led to other variables becoming statistically significant, or itself became significant. Thus although the criteria for retaining variables included significance at the 5% level, a variable may be included in the final model even if it was itself statistically non-significant. To prevent over-adjustment for puberty, Tanner score[364] was not included in the models. However, in sensitivity analyses models were refit containing either Tanner score for puberty as a covariate, or an interaction between age and sex in order to investigate puberty at recruitment. Models were investigated to ensure that missing data had no substantial impact.

2.8.4 Kaplan-Meir estimates

Kaplan-Meir estimates[372] were used to calculate the probability of emergence of a specific outcome (e.g. body fat alterations, metabolic abnormality etc.) over follow-up time, by subject characteristic (Table 2-1). Log-rank tests were used to formally test for differences in survival probabilities between groups.

Both maximum HIV-related clinical status, and nadir level of immunosuppression were updated, if necessary, to reflect any changes that occurred during follow-up. Additional variables were constructed to investigate time-updated potential risk factors. Detectable viral load during follow-up was defined as the occurrence of viral load >50copies/ml at any study visit at or following recruitment. Similarly CDC-defined HIV symptoms during follow-up was defined as the diagnosis of class B or C (Section B.2 in Appendix B) at recruitment or any subsequent study visit, and CDC-defined immunosuppression during follow-up was defined as evidence of immunosuppression at recruitment (by age thresholds: Section B.3 in Appendix B) or any subsequent study visit.

Hazard plots were used to ascertain each factor's suitability to be included in Cox proportional hazard survival models.

2.8.5 Cox proportional hazard survival models

Cox proportional hazard models[373] were used to investigate the factors associated with the incidence of LS during the follow-up period. Both univariable and multivariable models were investigated. As with multivariable logistic models, multivariable Cox models were either intuitively adjusted, or fitted including variables that were selected by stepwise selection (using the same method as outlined above in Section 2.8.3). Furthermore, all multivariable Cox models were adjusted for age and duration of ART use at recruitment, and included a random effect (frailty term) in the intercept to account for unobserved variables in different clinical sites.

Models were refit containing either Tanner score for puberty as a covariate, or an interaction between age and sex in order to investigate puberty at recruitment: none of these terms reached statistical significance in any model, and thus these models were discarded.
The proportional hazards assumption for the Cox regression models was investigated by examining Schoenfeld residuals for each variable included in the model against follow-up time, and also performing the Therneau-Grambsch global test of non-proportionality[374]

2.8.6 Multilevel modelling

Repeated measures of serum concentration of lipids for each individual, collected over follow-up, were analysed by multilevel modelling[375,376]. These models investigate the relationship between explanatory variable(s) and the outcome by allowing for the correlation within clusters (i.e. repeated measures within the same subject) by modelling the variance and covariance between these measures. Residuals are split into two components; (i) specific to each subject which is constant over each measure, and (ii) specific to each subject at each measure. This can be summarized in Equation 1,

$$\boldsymbol{y_{ij}} = \boldsymbol{\beta X_i} + \boldsymbol{\vartheta_i} + \boldsymbol{\epsilon_{ij}} \,,$$

Equation 1

where $\boldsymbol{y_{ij}}$ is the outcome on the $\boldsymbol{j}$-th measure of the $\boldsymbol{i}$-th subject, $\boldsymbol{\beta}$ are the coefficient(s) of the matrix $\boldsymbol{X_i}$ of subject-specific explanatory variables in the regression model: $\boldsymbol{\beta X_i}$ is known as the linear predictor. Furthermore, $\boldsymbol{\vartheta_i}$ are the residuals specific to subject $\boldsymbol{i}$ over each measure (known as the random intercept of each subject), and $\boldsymbol{\epsilon_{ij}}$ are the residuals at measure $\boldsymbol{j}$ of subject $\boldsymbol{i}$ (the within-subject residual). Both $\boldsymbol{\vartheta_i}$ and $\boldsymbol{\epsilon_{ij}}$ are assumed to be normally distributed with mean = 0 and variances $\boldsymbol{\psi}$ and $\boldsymbol{\theta}$, respectively. Commonly, $\boldsymbol{\beta X_i}$ is known as the fixed effects, whereas $\boldsymbol{\vartheta_i}$ is known as the random effects part. Since the objective of multilevel models in this investigation is to examine the effects of LS on metabolic outcomes, a mixed

effects approach has been adopted: the correlation within clusters is modelled such that we can treat individuals and measures as sampled from respective populations, and thus inferences regarding $\boldsymbol{\beta}$ can be made to this wider population.

The model in Equation 1 is an example of a 2-level model. However, within this study repeated measures i.e. study visits (level 1) are obtained for each subject (level 2) and each subject is clustered within a number of clinical site (level 3). Thus outcomes were modelled using a 3-level model (Figure 2-5) as shown in Equation 2,

$$\boldsymbol{y_{ijk} = \beta X_{ij} + \vartheta_{ik}^{(2)} + \vartheta_{k}^{(3)} + \epsilon_{ijk},}$$

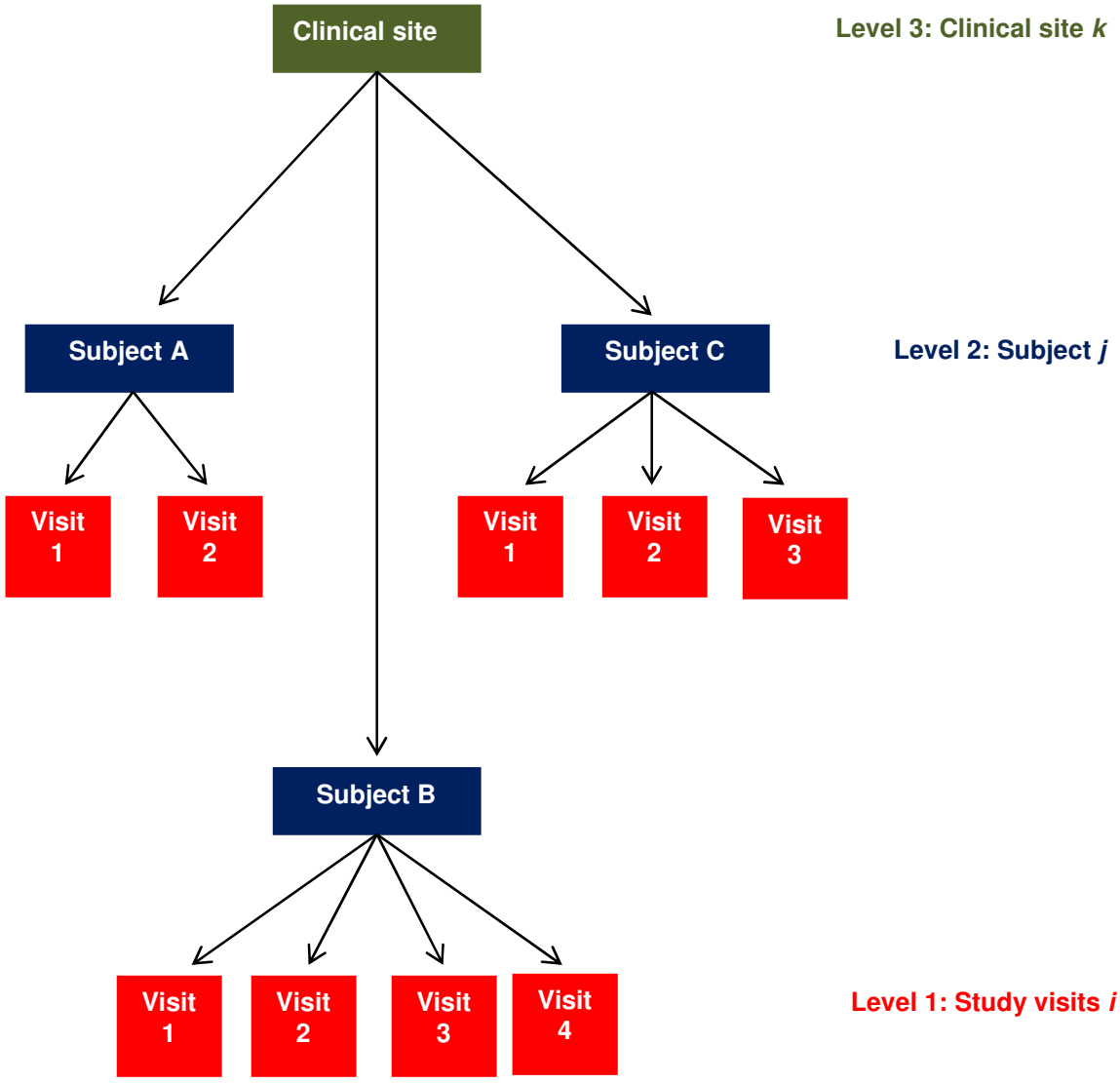
Equation 2

where $\boldsymbol{\vartheta_{ik}^{(2)}}$ is the random intercept for subject $\boldsymbol{j}$ at clinical site $\boldsymbol{k}$, and $\boldsymbol{\vartheta_{k}^{(3)}}$ is the random intercept for clinical site $\boldsymbol{k}$. The random effect for subject is nested within clinical site: it does not take the same value for an individual across a given clinical sites, but takes on a different value for each combination of subject and clinical site. The term $\boldsymbol{\epsilon_{ijk}}$ represents the random effect at the repeat-measure level within an individual: a departure from the individual effect within a clinical site. As subjects were recruited independently and there's no reason to expect any association between clinical sites, we assumed that the corresponding covariances determining the random effects were 0. As above, $\boldsymbol{\beta}$ represents the fixed effects parameters.

Multilevel modelling was used to investigate the relationship between LS and lipid outcomes (serum concentrations of total cholesterol, LDL-cholesterol, non-HDL cholesterol, HDL-cholesterol, and fasting triglyceride). All models had 3 levels, with subject clustered in clinical site included in the random intercept. Maximum likelihood estimation procedures were used.

Univariable models investigating all explanatory variables summarized in Table 2-1 were constructed: both recruitment variables and time-updated variables were investigated as appropriate. The covariates retained in the fixed effects for all multivariable models were age at recruitment, sex, and duration of ART use at recruitment, as possible confounding factors. Multivariable models were constructed by either including covariates by stepwise variable selection, or by intuitive selection, e.g. cholesterol and fasting triglyceride levels are known to be associated with sex and age, with further covariates chosen from results of previous regression models (Chapters 4- 6) also retained in all models (see Chapter 7 for details). As above the threshold to retain covariates in the stepwise selection procedure was 5%, However, a non-significant covariate could be retained if its inclusion caused a second covariate to become statistically significant or increased its effect size by >10%.

Figure 2-5: Multilevel structure of collected longitudinal data



Log ratio tests were conducted to examine the effect of inclusion of the 2nd level and 3rd level. For each model two tests were conducted; (i) comparing the final 3-level model with the null model with no random effects, and (ii) comparing the 3-level model with the 2-level model with intercept random effects for individual. Twice the difference in log likelihoods of the two models was compared to the appropriate value of the χ^2 distribution (against 2 degrees of freedom for comparisons with the null model, and 1 degree of freedom for comparison with the 2-level model). A 5% significance threshold was set to determine whether inclusion of the random effect terms improved the fit of the model to the data.

The normal assumption in both the level 2 and level 3 residuals in the final 3-level models was checked by assessing linearity in quantile-quantile (QQ) plots of the inverse normal distribution against standardised residuals. The normal assumption of the random effects was assessed in the same way.

2.9 Role of the researcher

The European Paediatric HIV and Lipodystrophy Study was set up based on a research concept formulated by Claire Thorne, Alessandra Vigano and Tessa Goetghebuer. All three designed the data collection tools. Alessandra Vigano, Tessa Goetghebuer, and Magdalena Marczynska oversaw recruitment and data collection across sites in Italy, Belgium and Poland respectively. Recruitment into the study occurred during 2007 and 2008. I joined the study team in January 2009, having secured a Medical Research Council PhD studentship to investigate LS in HIV-infected children and adolescents.

I was responsible for the coordinating the data entry in London of the paper questionnaires from sites in Belgium and Poland, entering some of these forms myself and overseeing data entry by an administrator for the remaining patients. I co-ordinated the data flow from sites in Belgium and Poland, and the electronic transfer of the Italian datasets to London. I carried out data cleaning, liaised with local clinicians with respect to data queries (i.e. where data were missing, ambiguous, or conflicting) and was responsible for overall data management for the study.

I performed consistency and other validity checks on the data to identify data items that were not feasible. I also managed the final dataset before conducting all the analyses presented in this thesis, with statistical advice provided by Mario Cortina Borja.

2.9.1 Dissemination of analyses from this thesis

I am the first author of one original paper, reporting findings from this thesis, published in a peer reviewed journal. I have also had three conference abstracts published. A second original paper has been prepared: I am first author and this paper will be submitted for consideration for publication in a peer reviewed journal in autumn 2012. Documents are included in the Appendix A.

Peer reviewed journal article

- Alam N, Cortina-Borja M, Goetghebuer T, Marczynska M, Vigano A & Thorne C. Body fat abnormality in HIV-infected children and adolescents living in Europe: Prevalence and risk factors. *JAIDS* 2012; 59(3): 314-324

Oral presentations

- 18th International AIDS Conference
    - *Risk factors for metabolic abnormality in a European cohort of HIV-infected children and adolescents*

- Presented in Vienna, Austria in July 2010

- 2nd International Workshop on HIV Pediatrics
  - *Risk factors for metabolic abnormality in a European cohort of HIV-infected children and adolescents*
  - Presented in Vienna, Austria in July 2010

Poster presentations

- 54th Society of Social Medicine Scientific Conference
  - *Patterns of antiretroviral therapy in a European study of HIV-infected children and adolescents*
  - Presented in Belfast, United Kingdom in September 2010
- 11th International Workshop on Adverse Drug Reactions & Co-morbidities
  - *Risk factors for body fat redistribution in a European cohort of HIV infected children and adolescents*
  - Presented in Philadelphia, USA in November 2009
- 16th Conference on Retroviruses and Opportunistic Infections (CROI): (***Non-lead author)***
  - *Active surveillance of body fat changes and metabolic abnormalities in HIV-infected children and adolescents in Europe: first round results*
  - Presented in Montreal, Canada in February 2009

3. Prevalence of lipodystrophy at recruitment: characterization of body fat alterations and metabolic abnormalities

3.1 Objectives

The main objective of this chapter is to describe the characteristics of subject participants at recruitment, and explore the manifestation of LS. Specific objectives include;

- Description of missing data in both explanatory and outcome variables
- To describe the socio-demographic and physiological characteristics of study participants at recruitment.
- Outline the ART profile of subjects and examine the characteristics of those who are ART naïve.
- Estimate the prevalence of LS at recruitment, investigating the proportions defined by body fat alterations (specifically lipoatrophy or lipohypertrophy) and metabolic abnormality (specifically hypercholesterolemia, fasting hypertriglyceridemia, and glucose intolerance).
- Explore the manifestation of fat redistribution with reference to lipoatrophy and lipohypertrophy at specific body locations, and accompanying severity.
- Investigate the distributions of covariates (body mass index: BMI, waist/hip ratio, total cholesterol, LDL-cholesterol, non-HDL-cholesterol, HDL-cholesterol, fasting triglyceride, fasting insulin and fasting glucose) across sexes and age bands, and body fat alterations and metabolic abnormality outcomes.

3.2 Methods

Analyses presented in this chapter are based on enrolment data, i.e. collected in form A (Section B.4 in Appendix B) collected at the time of recruitment. Missing data were excluded from both the numerator and denominator of estimates of prevalence.

3.2.1 Definitions

When investigating gestational characteristics of subjects, full term gestational age was defined as 37 completed weeks, and low birth weight as less than 2500g. The CDC defines being *risk of becoming overweight* as having sex- and age-specific BMI of between the 85th and 95th percentile, being *overweight* a BMI ≥ 95th percentile, and being *underweight* as a BMI ≤ 5th percentile[377,378]: These percentiles were conceived with reference to data collected in a representative sample of the US population in the National Health and Nutrition Examination Survey (NHANTES). The CDC percentiles were used to estimate prevalences of being at risk of being underweight or being overweight among subjects with lipoatrophy and those with lipohypertrophy.

3.2.2 Comparison of medians and proportions

Medians were compared with the Wilcoxon-Mann-Whitney and Kruskall-Wallis tests, while comparisons of proportions were conducted using χ^2 tests: the level of significance was set at 5%.

3.2.3 Investigation of antiretroviral naïve subjects

In order to investigate the participants who were ART-naïve at recruitment, subset analyses restricted to these subjects was conducted. To identify both the direction and magnitude of factors associated with ART-naivety, logistic regression models were constructed with a binary outcome (ART-naïve/not ART-naïve), and postulated risk factors as explanatory variables.

3.2.4 Exploration of changes in anthropometric measures and serum lipids

Serum cholesterol, triglyceride and fasting insulin vary with increasing age and between sexes[379-382]. The same is true of BMI: both the CDC and WHO providing age and sex dependent *z*-scores[383,384]. The CDC also provide *z*-scores for weight-by-height[383]. Thus, age and sex standardized (within the population) measures of these variables (Box 3-1) were calculated for males and females i.e. the individual values of the variable were standardized against the mean value of the value within each age and gender group, and errors calculated. Differences in medians of each variable in 2-year age groups were investigated in order to investigate whether differences occurred during puberty. Analysis with a single statistical model incorporating age and gender would result in the estimation of effect of age across the whole of adolescence: thus this approach was not used in these descriptive analyses. To explore patterns of joint variation in continuous variables in the study population at recruitment: the method of locally weighted scatterplot smoothing was used, as described by Cleveland in 1979[366].

Box 3-1: Outcome measures investigated at recruitment

Anthropometric measures

Body mass index (BMI)

Waist/hip ratio

Metabolic measures

Cholesterol

Fasting triglyceride

Fasting insulin

Fasting glucose

Locally weighted smoothing

Since the size of this study population is less than 500 subjects, the approach of locally weighted nonparametric smoothing is convenient for examining the change in each of the variables listed up with increasing age for males, and for females[385].

Fractional polynomial modelling

To further explore the relationship between age at recruitment and each outcome measure stratified by sex, as illustrated in Box 3-1, fractional polynomial models were fitted. Since the data are cross-sectional, the object is not to determine a causal or longitudinal relationship, i.e. these models are not looking at how an individual's measures of a given characteristic change with increasing age. The smooth curves produced by these models allow inferences to be made regarding changes in these characteristics conditional on age.

Since fractional polynomials define regression models usually based on small range of transformations for the explanatory variables, there may be qualitative differences in the smooth curves corresponding to, for example individuals with lipodystrophy and those without for a given covariate. However, this is only indicative of a possible effect of LS: such conclusions cannot be made from cross sectional data. Nevertheless structural differences in fractional polynomial models may be informative in this descriptive analysis investigating possible alterations with regard to specific continuous characteristics is subjects with and without LS. However differences between groups may still be attributable to chance. Conclusions regarding the role of lipodystrophy, if any, in the relationship between age at recruitment and participant characteristics can be insightful but should be treated with caution.

Mean order-two models were then constructed for each measure stratifying by sex either with or without LS, i.e. 4 models for each measure. Reasons for using mean order two models are discussed in Chapter 2.

Anthropometric measures then had further models constructed for subjects with/without fat abnormality, and metabolic measures had further models constructed for subjects with/without metabolic abnormality.

Comparisons using median and standardized measures

Both crude and standardized measures of metabolic and anthropometric measures (Box 3-1) were plotted across age groups: 2-3, 4-5, 6-7, 8-9, 10-11, 12-13, 14-15, and 16-18 years. These age categories were used to examine age-related differences in LS outcomes with specific emphasis on differences that may occur during puberty. Both hypercholesterolemia and fasting hypertriglyceridemia were defined according to 1-year age bands in males above the age of 11 years, and females aged above the age of 9 years (Appendix B, Section B.2). However, two-year age bands were used in the comparisons presented in this chapter in order to circumvent the occurrence of categories with small numbers of subjects: for example, there were 5 females aged 2 years old, and 13 males aged 4 years.

Age and gender standardized estimates of BMI and waist/hip ratio were compared using Wilcoxon-Mann-Whitney and Kruskall-Wallis tests between subjects (a) with and without LS, (b) with and without body fat disorder, and (c) with and without metabolic abnormality.

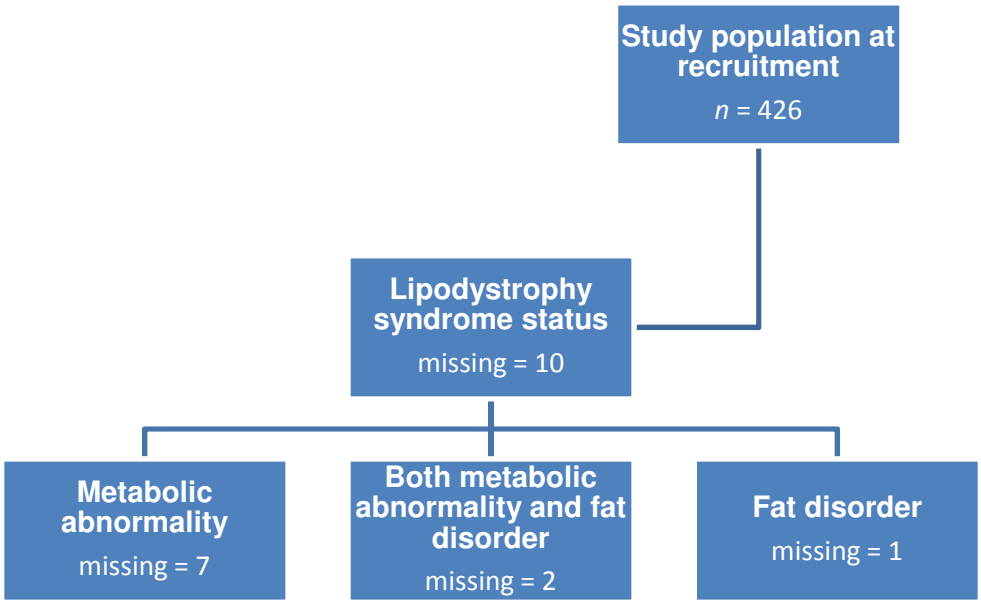
Median total cholesterol, LDL-cholesterol, non-HDL cholesterol, HDL-cholesterol, fasting triglyceride, fasting insulin, and fasting glucose were compared across age groups between subjects according to a) b) and c) as in the previous paragraph. However, each of these outcomes, with the exceptions of fasting insulin and fasting glucose are direct components in the definitions of metabolic abnormality and lipodystrophy syndrome. The objective of these comparisons was to look at the relationship between body fat alterations and metabolic outcomes, and to determine whether there were specific ages where the metabolic abnormality presented. Thus although the interpretation of differences seen with metabolic abnormality and lipodystrophy syndrome are confounded, an implication of the role of age may be postulated.

3.3 Missing data

3.3.1 Lipodystrophy outcomes

Data on LS outcomes was missing for 10 subjects: 7 subjects were missing data on metabolic abnormality but not body fat alterations, 1 subject was missing data on body fat alterations but not metabolic abnormality, and 2 subjects were missing data on both fat disorder and metabolic abnormality (Figure 3-1). Of these 10 subjects, 7 were attending 5 hospitals in Italy. Half of the subjects with missing LS outcome data were male, and 6 were aged ≥15 years (all but one of these being female). With regard to ART use at recruitment, 3 were currently not on ART and 1 was also missing this information.

Figure 3-1: Missing data with regard to lipodystrophy outcomes



3.3.2 Metabolic and physiological variables

Table 3-1 shows the missing data related to each outcome: 1.5% of BMI, 25% of waist hip ratio, 1.0% total cholesterol, 17%% LDL cholesterol, 15% non-HDL cholesterol, 15% HDL cholesterol, 1.7% fasting insulin, and 1% fasting glucose were missing across each of the lipodystrophy outcomes. In contrast, no subjects with data on fasting triglyceride, had missing data for any of these variables.

The proportions of missing values of HDL-cholesterol and LDL-cholesterol measures may lead to misestimating differences in levels of these measures between subjects with a given outcome and those without. However, there were no significant associations between neither metabolic

abnormality nor fasting hypertriglyceridemia with missing data for any of the following measures: total cholesterol, LDL-cholesterol, HDL-cholesterol, non-HDL-cholesterol, fasting triglyceride, fasting insulin, or fasting glucose. A significant association was found between hypercholesterolemia and missing insulin values. Despite this, it is unlikely that a diagnosis of some form of metabolic abnormality led to a greater likelihood of more metabolic tests in children with metabolic abnormality compared to those conducted in those children without.

The only body fat alterations outcome which was significantly associated with missing data (in metabolic measures) was lipoatrophy, which was associated with missing values of HDL-cholesterol, LDL-cholesterol, non-HDL-cholesterol and insulin ($p < 0.05$ for all tests)[g]. Neither missing data in waist/hip ratio nor BMI was significantly associated with any body fat alterations outcome. Missing values for waist/hip ratio also showed a significant association with ethnicity. This was driven by country differences in ethnicity: all subjects in Poland were of white ethnicity, and no subjects in Belgium were missing waist/hip ratio (Appendix C, Table C-1).

Missing data in HDL-cholesterol, LDL-cholesterol and non-HDL-cholesterol were associated with country of residence. This may have been driven by a number of Italian hospitals which did not have missing data for any of these variables (Como, Genova and S. Paolo). Furthermore, missing data in BMI and waist/hip ratio were also significantly associated with country of residence, due to the one Polish hospital participating in the study not collecting this data. Indeed, the high levels of missing data for cholesterol (in comparison to missing data in other variables), may have led to no significant differences being seen in median levels between subjects with and those without body fat alterations.

3.3.3 Explanatory variable data

Data collection forms were designed so clinicians would enter patient past and current ART therapy as free text. Thus, as no direct questions were asked regarding specific ART, evaluation of missing data pertaining to HIV treatment was not possible. However, direct dichotomous or categorical questions were asked about other explanatory variables (Table 3.2). Only a small proportion of data was missing, and most explanatory variables were >94% complete. However recruitment and maximum CDC-defined clinical status had up to 16% and 8% missing. Furthermore, Tanner score for puberty had >16% missing data for each LS outcomes. However, Tanner score was not used in any of the main analyses presented in this thesis (only being used in sensitivity analyses.

[g] No analyses presented in this thesis focused on the relationship between lipoatrophy and these specific lipid subgroups. It is possible that analyses investigating body fat alterations and these cholesterols may be affected by this missing data. As only 34.7% of subjects with body fat alterations were characterized by lipoatrophy-alone, the effect of this potential bias is difficult to gauge.

Table 3-1: Missing physical and metabolic data by lipodystrophy outcome

	Total missing	Lipodystrophy		Fat alterations		Lipohypertrophy		Lipoatrophy		Metabolic abnormality		Hyper-cholesterolemia		Fasting hyper-triglyceridemia		Glucose intolerance	
	n	*%*	*n/N*	*%*	*n/N*	*%*	*n/N*	*%*	*n/N*	*%*	*n/N*	*%*	*n/N*	*%*	*n/N*	*%*	*n/N*
BMI	8	*1.4*	6/416	*1.4*	6/422	*1.4*	6/421	*1.4*	6/420	*1.4*	6/415	*1.4*	6/423	*1.4*	6/418	*1.4*	6/417
Waist/hip ratio	109	*25.0*	104/416	*24.9*	105/422	*24.9*	105/421	*24.5*	103/420	*25.3*	105/415	*25.1*	106/423	*24.9*	104/418	*25.4*	106/417
Total cholesterol	7	*0.5*	2/416	*1.0*	4/422	*1.0*	4/421	*1.0*	4/420	*0.2*	1/415	*1.0*	4/423	*0.0*	0/418	*1.0*	4/417
LDL cholesterol	77	*16.6*	69/416	*17.5*	74/422	*17.3*	73/421	*17.6*	74/420	*16.4*	68/415	*17.5*	74/423	*16.5*	69/418	*17.0*	71/417
Non-HDL cholesterol	66	*14.2*	59/416	*14.9*	63/422	*15.0*	63/421	*15.0*	63/420	*14.0*	58/415	*15.0*	63/423	*14.1*	59/418	*14.4*	60/417
HDL cholesterol	66	*14.2*	59/416	*14.9*	63/422	*15.0*	63/421	*15.0*	63/420	*14.0*	58/415	*14.9*	63/423	*14.1*	59/418	*14.4*	60/417
Fasting triglyceride	8	*0.0*	0/416	*0.0*	0/422	*0.0*	0/421	*0.0*	0/421	*0.0*	0/423	*0.0*	0/423	*0.0*	0/418	*0.0*	0/417
Fasting insulin	182	*1.7*	7/416	*1.7*	7/422	*1.4*	6/421	*1.7*	7/420	*1.7*	7/415	*1.7*	7/423	*1.7*	7/418	*1.7*	7/417
Fasting glucose	16	*0.05*	2/416	*0.05*	2/422	*0.10*	4/421	*0.05*	2/420	*0.05*	2/415	*0.05*	2/423	*0.05*	2/418	*0.05*	2/417

Missing data for outcomes: Lipodystrophy syndrome 2.3% (10/426), fat alterations 1.0 (4/426), lipohypertrophy 1.1% (5/426), lipoatrophy 1.4% (6/426), metabolic abnormality 2.6% (11/426), hypercholesterolemia 1.0% (3/426), fasting hypertriglyceridemia 0.0% (0/426), and glucose intolerance 2.1% (9/426)

Table 3-2: Missing explanatory variable data by lipodystrophy outcome

	Total missing	Lipodystrophy		Fat redistribution		Lipohypertrophy		Lipoatrophy		Metabolic abnormality		Hyper-cholesterolemia		Fasting hyper-triglyceridemia	
	n	%	*n/N*	%	*n/N*	%	*n/N*	%	*n/N*	%	*n/N*	%	*n/N*	%	*n/N*
Demographic factors															
Gender	11	2.98	7/235	3.41	6/176	2.61	3/115	4.35	5/115	4.63	5/108	7.14*	4/56	0.00	0/71
Ethnicity	17	4.68	11/235	5.68	10/176	2.61	3/115	5.88***	10/170	3.70	4/108	7.14	4/56	1.41	1/71
Country of residence	0	0.00	0/235	0.00	0/176	0.00	0/115	0.00	0/117	0.00	0/108	0.00	0/56	0.00	0/71
HIV Clinical factors															
CDC immune stage at recruitment	0	0.00	0/235	0.00	0/176	0.00	0/115	0.00	0/117	0.00	0/108	0.00	0/56	0.00	0/71
Nadir CDC immune stage at recruitment	0	0.00	0/235	0.00	0/176	0.00	0/115	0.00	0/117	0.00	0/108	0.00	0/56	0.00	0/71
CDC clinical stage at recruitment	48	13.2	31/235	13.07	23/176	13.04	15/115	14.53	17/117	12.96	14/108	16.07	9/56	11.27	8/71
Maximum CDC clinical stage	24	5.11	12/235	4.55	8/176	1.74*	2/115	6.84	8/117	5.56	6/108	7.14	4/56	4.24	3/71
HIV-RNA concentration at recruitment	2	0.42	1/235	0.57	1/176	0.87	1/115	0.00	0/117	0.93	1/108	0.00	0/56	1.41	1/71
Mode of HIV acquisition	17	3.83	9/235	4.55	8/176	3.48	4/115	4.27	5/117	4.63	5/108	5.36	3/56	0.00	0/71
Other factors															
Hepatis C co-infection	10	1.70	4/235	1.70	3/176	0.87	1/115	1.71	2/117	0.92	1/108	1.79	1/56	1.41	1/71
Tanner score for puberty	52	12.34	29/235	14.20	25/176	13.04	15/115	13.68	16/117	9.26	10/108	5.34	3/56	11.27	8/71
BMI at recruitment	8	1.28	3/235	1.70	3/176	2.61	3/115	1.71	2/117	0.00	0/108	0.00	0/56	0.00	0/71

χ^2 test for difference in proportion of missing data with/without outcome * 0.01 ≤ *p* < 0.05, **0.001 ≤ *p* < 0.01, and * *p* ≤ 0.001**

Statistically significant associations between missing data and LS outcomes were seen only in three situations: (i) maximum CDC-defined clinical status with lipohypertrophy; p = 0.039, (ii) ethnicity with lipoatrophy; p = 0.004 and (iii) gender with hypercholesterolemia; p = 0.022. In the case of Maximum clinical status, missing data was seen more commonly in subjects without lipohypertrophy (Table 3.3), which may lead to overestimation of an effect size on body fat gain associated with this variable. However, in the case of ethnicity with lipoatrophy and sex with hypercholesterolemia, missing data was more common in subjects with the respective outcome: this may lead to bias in estimation of any associated effect size.

Table 3-1: Comparison of missing data in explanatory variables where there was a significant difference in proportions with/without specific outcomes

Explanatory variable	Outcome	Without outcome		With Outcome		*p*- value
		%	n	%	n	
Maximum clinical status	**Lipohypertrophy**	6.86	21	1.74	2	0.039
Ethnicity	**Lipoatrophy**	2.31	7	8.55	10	0.004
Sex	**Hypercholesterolemia**	1.91	7	7.14	4	0.022

Statistical test χ^2 test

3.4 Socio-demographic and gestational variables

Analysis was conducted on 426 subjects who were aged between 2 and 18 years at the time of recruitment. Table 3-4 illustrates the socio-demographic and HIV-related characteristics of participants at recruitment. Median age was 12.2 years (interquartile range, IQR: 9.0, 15.0). The largest ethnic group was of White ethnicity (*n*=285, 69.7%), with 26.2% of Black ethnicity (*n*=107). The majority of participants were patients from Italy (*n*=276, 69.7%): there was a significant association between ethnicity and country of residence (χ^2 test: $p<0.001$) with 82.7% of Belgian patients of Black ethnicity compared to 14.7% among Italian and no Polish participants.

Table 3-2: Distribution of baseline socio-demographic and HIV-related characteristics (*n*=426)

		2-6 years *n*=43	7-11 years *n*=117	12-18 years *n*=266	Whole cohort *n*=426
Demographic factors					
Gender	**Male**	21 (*50.0*)	50 (*43.9*)	130 (*50.2*)	201 (*48.4*)
	Female	21 *(50.0)*	64 (*56.1*)	129 (*49.8*)	214 (*51.6*)
Ethnicity	**Black**	10 (*24.4*)	30 (*27.3*)	67 (*26.0*)	107 (*26.2*)
	White	29 (*70.7*)	73 (*66.3*)	183 (*70.9*)	285 (*69.7*)
	Other	2 (*4.9*)	7 (*6.4*)	8 (*3.1*)	17 (*4.2*)
Country of residence	**Belgium**	2 (*4.7*)	20 (*17.1*)	62 (*23.3*)	84 (*19.7*)
	Italy	26 (*60.5*)	76 (*65.0*)	174 (*65.4*)	276 (*64.8*)
	Poland	15 (*34.9*)	21 (*17.9*)	30 (*11.3*)	66 (*15.5*)
HIV clinical factors					
CDC immune stage at recruitment	**Stage 1**	27 (*62.8*)	101 (*86.3*)	196 (*73.7*)	324 (*76.1*)
	Stage 2	14 (*32.6*)	14 (*12.0*)	56 (*21.1*)	84 (*19.7*)
	Stage 3	2 (*4.7*)	2 (*1.7*)	14 (5.*3*)	18 (*4.2*)

		2-6 years *n*=43	7-11 years *n*=117	12-18 years *n*=266	Whole cohort *n*=426
Nadir CDC immune stage	**Stage 1**	14 (*32.6*)	65 (*55.7*)	81 (*30.5*)	160 (*37.6*)
	Stage 2	22 (*51.2*)	40 (*34.2*)	117 (*44.0*)	179 (*42.0*)
	Stage 3	7 (*16.3*)	12 (*10.3*)	68 (*25.6*)	87 (*20.4*)
CDC clinical stage at recruitment	**N+A**	32 (*89.0*)	94 (*93.1*)	220 (*91.3*)	346 (*91.5*)
	B	2 (*5.6*)	6 (*5.9*)	13 (*5.4*)	21 (*5.6*)
	C	2 (*5.6*)	1 (*1.0*)	8 (*3.3*)	11 (*2.9*)
Maximum CDC clinical stage	**N+A**	18 (*43.9*)	59 (*53.2*)	93 (*37.2*)	170 (*42.3*)
	B	14 (*34.2*)	31 (*27.9*)	98 (*39.2*)	143 (*35.6*)
	C	9 (*22.0*)	21 (*18.9*)	59 (*23.6*)	89 (*22.1*)
HIV-RNA at recruitment	**≤50 copies/ml**	29 (*67.4*)	78 (*66.7*)	139 (*52.7*)	246 (*58.0*)
	>50 copies/ml	14 (*32.6*)	39 (*33.3*)	125 (*47.4*)	178 (*42.0*)
Mode of HIV acquisition	**Blood products**	0 (*0*)	2 (*1.8*)	5 (*2.0*)	7 (*1.7*)
	Vertical transmission	43 (*100*)	111 (*98.2*)	246 (*97.2*)	400 (*97.8*)
	Other	0 (*0*)	0 (*0*)	2 (*0.8*)	2 (*0.5*)
Other factors					
Hepatitis C co-infection	**No co-infection**	39 (*95.1*)	111 (*96.5*)	241 (*93.0*)	391 (*94.0*)
	Co-infection	2 (*5.0*)	4 (*3.5*)	19 (*7.3*)	25 (*6.0*)

		2-6 years *n*=43	7-11 years *n*=117	12-18 years *n*=266	Whole cohort *n*=426
Tanner score for puberty	**I**	37 (*97.4*)	87 (80.6)	11 (4.8)	135 (*36.1*)
	II-IV	1 (*2.6*)	21 (19.4)	123 (53.9)	145 (*38.8*)
	V	0 (0)	0 (0)	94 (41.2)	94 (*25.1*)
BMI (kg/m^2)		16.01 (14.38, 17.09)	16.49 (15.07, 18.94)	19.73 (17.69, 22.57)	

CDC-defined immune status - stage 1: no immunosuppression, stage 2: moderate immunosuppression, stage 3: severe immunosuppression. CDC-defined clinical status - N + A: no symptoms/asymptomatic, B: moderate symptoms, C: severe symptoms.

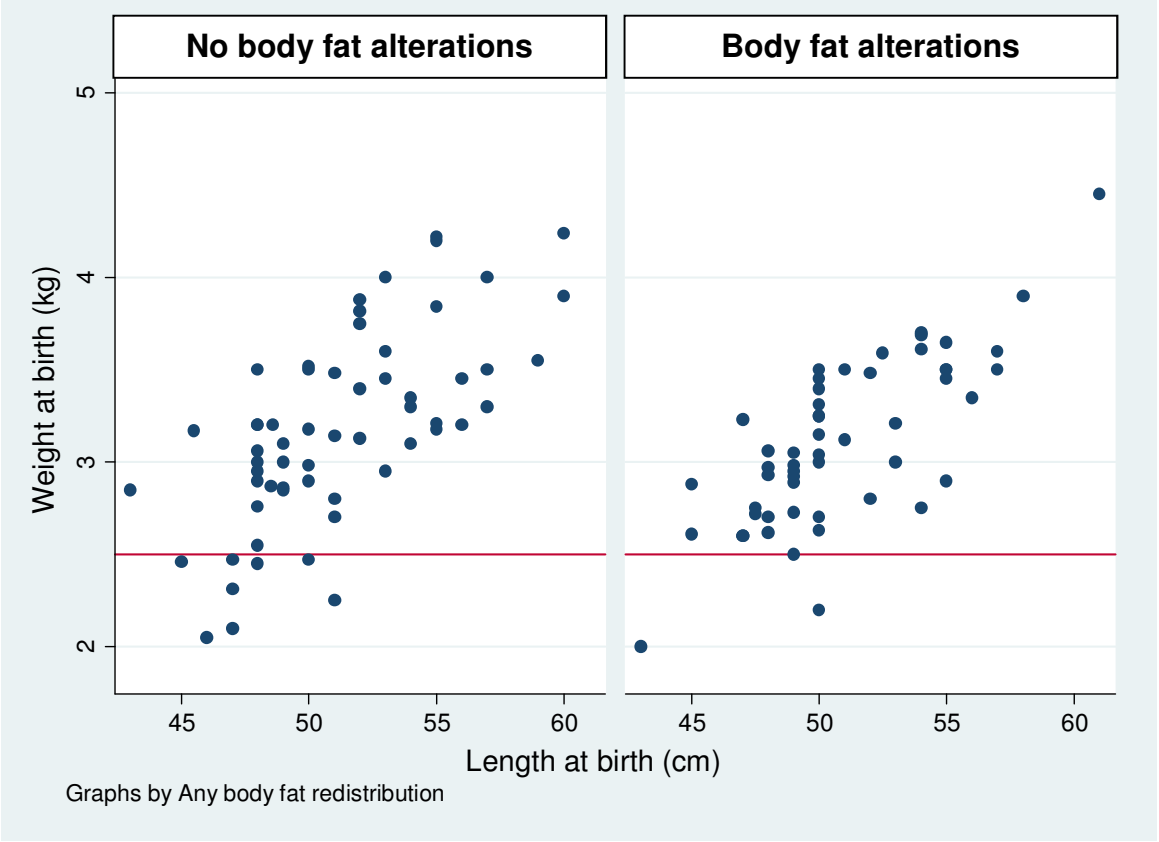
Data on gestational age at birth was missing for 164 subjects; of the remaining 264 subjects, 43 (16.3%) were born preterm. Over two-thirds of the study population (*n* = 286) had data on birth weight. The prevalence of low birth weight was 22.0% (63/286). The relationship between gestational age at birth and birth weight is illustrated in Figure 3-2, with similar patterns seen in males and females.

Figure 3-2: Relationship between gestational age and birth weight in males (*n* = 116) and females (*n* =121)

Vertical line indicates gestation of 37 weeks; horizontal line indicates birth weight of 2500g

The distribution of birth length and birth weight between subjects with/without body fat alterations at enrolment was similar (among subjects who had reached full gestational age of 37 weeks) is shown in Figure 3-3. The distribution among children with body fat alterations appears to be wider, with a lesser weight for a given length compared to subjects without fat disorder at recruitment. However, regression of body length on body weight in these two groups resulted in similar estimates for the intercept and slope, but the sum of mean squares of the variance was a third greater in body fat alteration group compared to the no fat alteration group (Appendix C, Table C-2).

Figure 3-3: Relationship between birth weight and birth length in subjects who were born at gestational age ≥37 weeks: between subjects without body fat alterations (*n* = 59) and those with body fat alterations (*n* = 53) at recruitment.



Only subjects who had been born at a gestational age ≥37 weeks shown. Median follow-up time: 11.8 years (IQR: 8.5, 14.3)) Horizontal line denotes birth weight of 2500g

3.5 Viral and clinical variables

Only 9 subjects (2.2%) did not acquire HIV through vertical transmission. Of the 7 participants who acquired HIV through blood products, five were born in Romania, all aged between 16 and 18 years. The remaining two subjects were born in the Ukraine and Poland, and both were 8 years old.

At recruitment, most participants showed no evidence of immunosuppression, with 76.1% (*n* = 324) at CDC stage 1 (Table 3-4). However almost two thirds (*n* = 266, 62.4%) of the cohort had experienced some form of immunosuppression. One fifth (*n* = 89, 20.9%) of the study population had experienced the most advanced CDC-defined clinical stage at some point up to enrolment, although 80% (*n* = 346, 80.1%) were asymptomatic at this time. Indeed, at recruitment, most participants were either asymptomatic or had only mild symptoms, showed no immunosuppression and had undetectable viral load (>58% across all age groups).

Of note, 25.6% (68/266) of adolescents (aged ≥12 years) had previously experienced severe immune-suppression, with 26.3% (59/250) having experienced severe clinical symptoms: 32 subjects aged ≥12 years had experienced both severe clinical symptoms and severe immunosuppression. Of the 364 subjects on ART at recruitment, over one-third (*n* = 131, 36.0%) had detectable viral load (1 subject was missing data on viral load): 44 of these subjects had either CDC-defined symptoms or evidence of immunosuppression.

3.6 History of antiretroviral therapy

The majority of participants (*n* = 364, 85.4%) were currently being treated with ART at recruitment: 29 subjects were ART naïve with no current or previous use. A further 33 subjects had ART history missing and so were excluded from calculation of prevalence of treatment. There were no subjects who had previously been on ART, but had discontinued ART use completely by the time of recruitment. The median age at initiation of ART was 5.2 years (Table 3-5). There was a significant difference in median age at first ART with increasing age category (Mann-Whitney-Wilcoxon test: $p < 0.001$) with older children starting ART at older ages. The median duration of ART at recruitment was 5.8 years (IQR: 2.6, 9.9).

Table 3-3: Median age (interquartile range) at initiation of ART and median ART duration at recruitment

	Age group						Whole cohort	
	2-6 year olds		7-11 year olds		12-18 year olds			
	Median (IQR)	N	Median (IQR)	*n*	Median (IQR)	*n*	Median (IQR)	*n*
Age at first ART (years)	2.78 (0.84, 4.21)	30	4.24 (1.99, 7.39)	90	6.21 (2.52, 9.77)	210	5.20 (2.20, 8.80)	330
Duration of ART (years)	1.48 (0.69, 3.13)	30	3.98 (1.41, 6.77)	92	7.54 (4.28, 11.49)	211	5.80 (2.60, 9.90)	333

Kruskall-Wallis test for equality of medians: age at first ART (p <0.001): duration of ART (p < 0.001)

Figure 3-4 illustrates the pattern of common ART regimens used at the time of recruitment: the most popular form of cART at the time of recruitment was PI-based HAART, followed by NNRTI-based HAART, while 14 subjects (4%) were treated with triple class therapy (i.e. at least one each of PI, NRTI and NNRTI). Of note, a small proportion (8%) of the study population was treated with NRTI mono-therapy.

Figure 3-4: Distribution of common combinations of antiretroviral therapies and NRTI-based mono-therapy at the time of recruitment (*n* = 364).

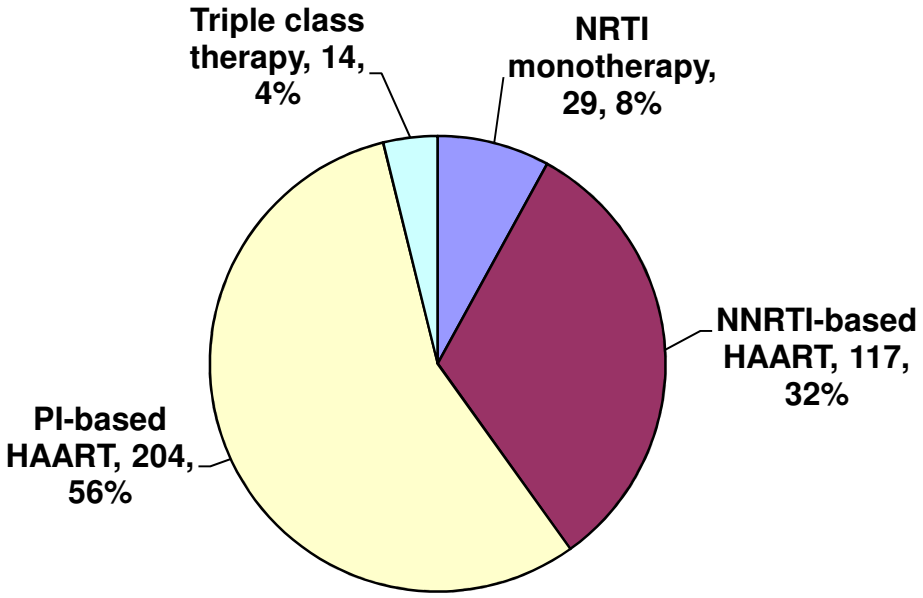


Table 3-6 shows the distribution of class of ART by both ever-use and use at the time of recruitment (current-use). All but two of the 364 subjects on ART (*n* = 362/393: 92% of all subjects whose ART status was known) at recruitment were being treated with NRTIs. The remaining two subjects were aged 15.4 years and 16.6 years, and on a NRTI-sparing regimen consisting of NNRTI and PI at recruitment (specifically efavirenz with fosamprenavir and lopinavir/ritonavir, and nevirapine with lopinavir/ritonavir respectively), and had both been treated by NRTI in the past. The second most popular class was PI in all age groups except the 7-11 year olds. Over half of participants (55.5%, *n* = 218 were currently on PIs, while one third (33.8%, *n* = 133) were on NNRTI. Only three participants (0.7%) had a history of FI use, falling to one participant (0.3%) by the time of recruitment.

Table 3-4: Distribution of past and current class of ART use stratified by age in the cohort at recruitment (*n* = 393)

		Age group						Whole cohort	
		2-6 years		7-11 years		12-18 years			
		Ever	Current	Ever	Current	Ever	Current	Ever	Current
NRTI	Yes	39 (*90.7*)	34 (*89.5*)	106 (*90.6*)	100 (*90.1*)	251 (*94.7*)	228 (*93.4*)	396 (*93.2*)	362 (*92.1*)
	No	4 (*9.3*)	4 (*10.5*)	11 (*9.4*)	11 (*9.9*)	14 (*5.3*)	16 (*6.6*)	29 (*6.8*)	31 (*7.9*)
	Missing	0	5	0	6	1	22	1	33
NNRTI	Yes	13 (*30.2*)	7 (*18.4*)	65 (*55.6*)	46 (*41.4*)	167 (*63.0*)	80 (*32.8*)	245 (*57.6*)	133 (*33.8*)
	No	30 (*69.8*)	31 (*81.6*)	52 (*44.4*)	65 (*58.6*)	98 (*37.0*)	164 (*67.2*)	180 (*42.4*)	260 (*66.2*)
	Missing	0	5	0	6	1	22	1	33
PI	Yes	34 (*79.1*)	24 (*63.2*)	84 (*71.8*)	47 (*42.3*)	210 (*79.2*)	147 (*60.2*)	328 (*77.2*)	218 (*55.5*)
	No	9 (*20.9*)	14 (*36.8*)	33 (*28.2*)	64 (*57.7*)	55 (*20.8*)	97 (*39.8*)	97 (*22.8*)	175 (*44.5*)
	Missing	0	5	0	6	1	22	1	33
FI	Yes	0 (*0.0*)	0 (*0.0*)	0 (*0.0*)	0 (*0.0*)	3 (*1.1*)	1 (*0.4*)	3 (*0.7*)	1 (*0.3*)
	No	43 (*100.0*)	38 (*100.0*)	117 (*100.0*)	111 (*100.0*)	262 (*98.9*)	243 (*99.6*)	422 (*99.3*)	392 (*99.8*)
	Missing	0	5	0	6	1	22	1	33

Subjects on/not on a specific class of ART are given as percentage of number of subjects within that age group/whole cohort, i.e. column percentages for each class. Subjects where the ART history was unknown (*n* = 33) are excluded. NRTI: nucleoside reverse transcriptase inhibitor, NNRTI: non-nucleoside reverse transcriptase inhibitor, PI: protease inhibitor, and FI: fusion inhibitors.

Figure 3-5 and Figure 3-6 illustrate the distribution of ever-use and current use of specific ART drugs (Appendix C, Table C-3). The most commonly used NRTI, and the most commonly used drug overall, both historically and at recruitment was lamivudine. The second most common ever-used NRTI was zidovudine (*n* = 308, 72.5%), and the second most common NRTI in current use was abacavir (*n*= 118, 30.0%). While just over half of subjects had been exposed to stavudine at some point (*n* = 218, 51.3%), at recruitment only one in nine (*n* = 44, 11.2%) were using this drug. Lopinavir/ritonavir was the most common PI in both ever (*n* = 257, 60.47%) and current use (*n* = 197, 50.13%). Despite almost half (*n* = 204) of the study population having been exposed to nelfinavir, only one subject (0.25%) was currently on nelfinavir at the time of recruitment.

Only two NNRTIs were used by participants, with efavirenz used by almost one quarter (*n*=94) at recruitment, and nevirapine used by almost 10% (*n*=39). The sole FI in use in the cohort was enfuvirtide, with three participants (0.7%) with a history of its use, and one participant (0.3%) currently on enfuvirtide therapy at recruitment.

Figure 3-5: Distribution of ever-use of specific antiretroviral drugs

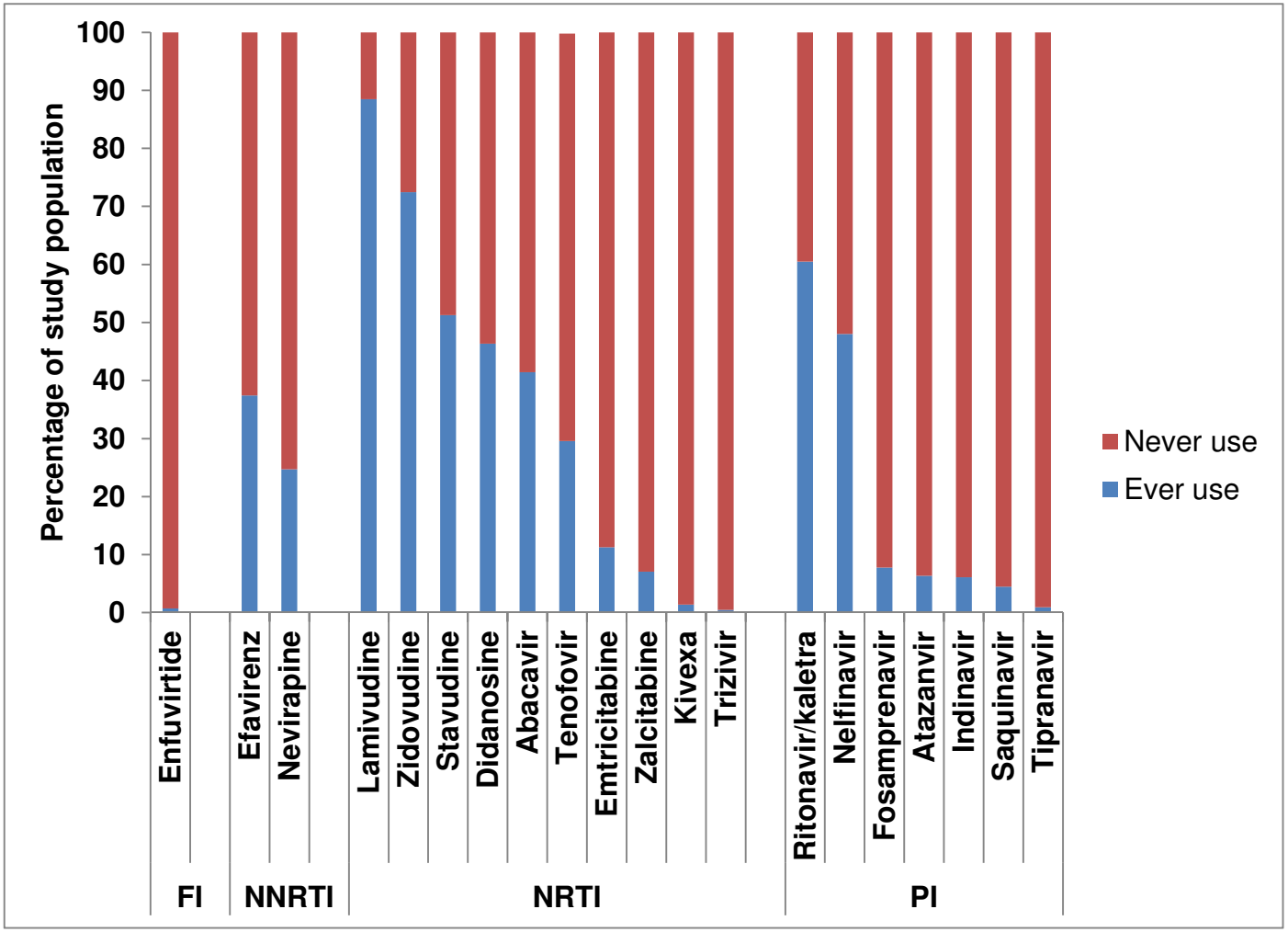
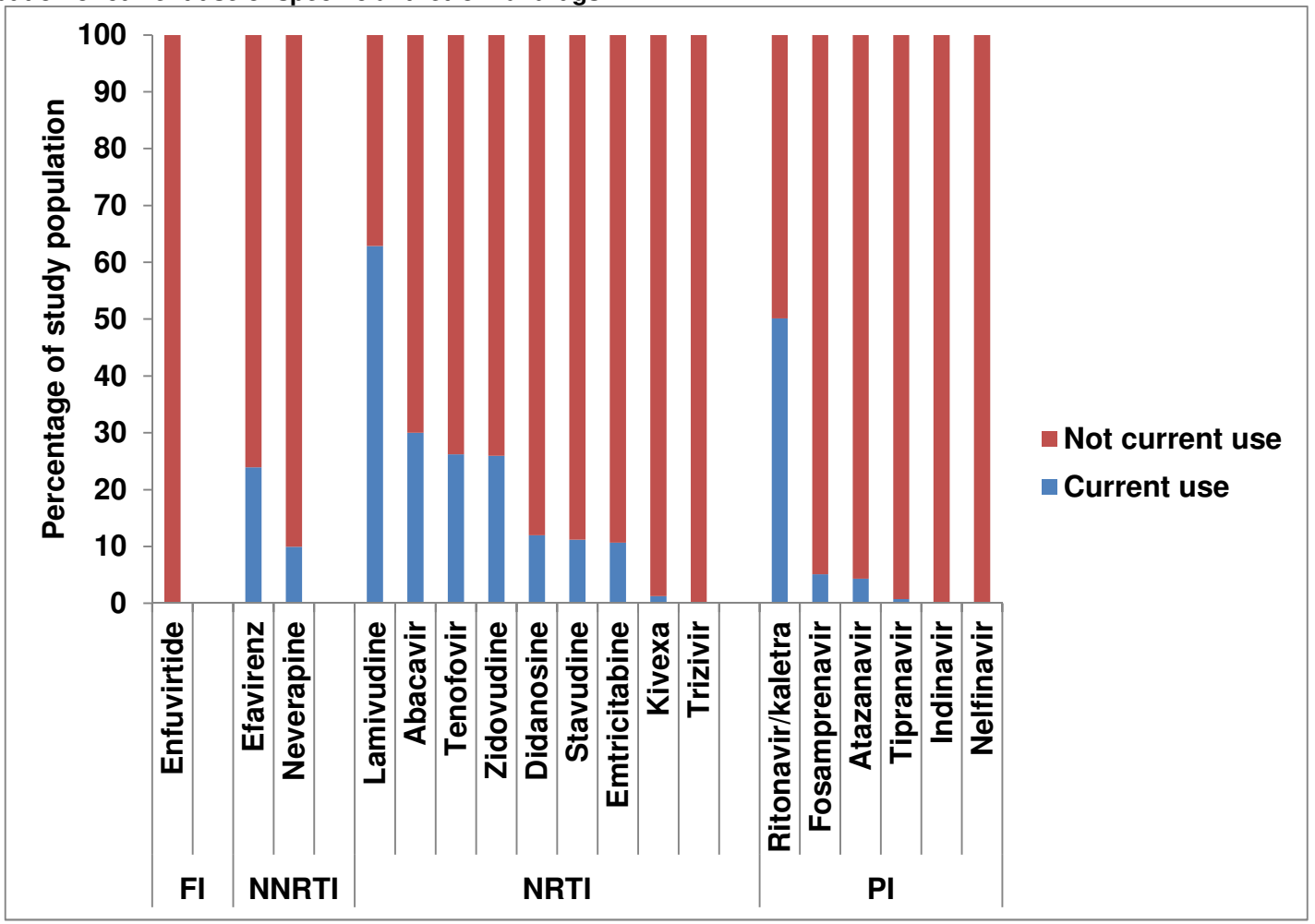


Figure 3-6: Distribution of current use of specific antiretroviral drugs



3.7 Antiretroviral naïve participants

Twenty-nine participants were ART-naïve; Table 3-7 illustrates their characteristics. Their median age was 10.48 years (IQR; 7.77, 15.26), which was lower than the median age in ART-treated subjects, although this difference was not significant (p = 0.465). Two-thirds of ART-naïve subjects were female, and the majority (n = 19) were from Italy.

Over 60% (n = 18) of these subjects showed either evidence of immune-suppression (n = 15) or HIV-related clinical symptoms (n = 3) at recruitment, and all but 4 had detectable viral load. At recruitment, 1 subject had serious clinical symptoms, and a further 3 subjects had severe immunosuppression. Furthermore, over three-quarters of ART-naïve subjects (n = 22) had a history of symptomatic disease and/or immunosuppression. Indeed being ART-naïve at recruitment was significantly associated with increased risk of current immuno-suppression in univariable analysis (Table 3-5). Furthermore, it was associated with a non-significant reduced risk of past clinical disease (p > 0.05). Multivariable models investigating factors associated with ART-naivety did not converge.

Of interest, 4 subjects who were ART-naïve had undetectable viral load at recruitment. Of these, 1 subject had history of moderate immunosuppression, 1 had history of severe immunosuppression, and 1 had history of severe CDC-defined clinical symptoms. Thus 1 ART-naïve subject had controlled viral suppression at recruitment, with no history of clinical symptoms and immunosuppression, i.e. was a long-term non-progressor.

Table 3-5: Characteristics of antiretroviral therapy naïve participants (*n* = 29)

		Prevalence		Univariable analyses			
		n	**%**	***n***	**OR**	**95% CI**	***p*-value**
Demographic factors							
Age(years)†		29	10.48* (7.77, 15.26)**	393	0.95	(0.86, 1.04)	0.291
Gender	**Male**	9	(33.3)	187	1		
	Female	18	(66.7)	197	1.99	(0.87, 4.55)	0.103
Ethnicity	**Black**	8	(29.6)	95	1		
	White	19	(70.4)	269	0.83	(0.35, 1.96)	0.665
	Other	0	(0.0)	-	-	-	-
Country of residence	**Italy**	19	(65.5)	264	1		
	Belgium	4	(13.8)	69	0.79	(0.26, 2.41)	0.684
	Poland	6	(20.7)	60	1.43	(0.55, 3.76)	0.465
HIV clinical factors							
CDC immune stage at recruitment	**Stage 1**	14	(48.3)	304	1		
	Stage 2	12	(41.4)	72	4.14	(1.83, 9.40)	0.001
	Stage 3	3	(10.3)	17	4.44	(1.14, 17.25)	0.031

		Prevalence		Univariable analyses			
		n	**%**	***n***	**OR**	**95% CI**	***p*-value**
Nadir CDC immune stage	**Stage 1**	10	(34.5)	142	1		
	Stage 2	17	(58.6)	166	1.51	(0.67, 3.40)	0.325
	Stage 3	2	(6.9)	85	0.32	(0.07, 1.49)	0.146
CDC clinical stage at recruitment	**N/A**	22	(88.0)	325	1		
	B	2	(8.0)	20	1.53	(0.33, 7.02)	0.584
	C	1	(4.0)	11	1.38	(0.17, 11.25)	0.765
Maximum CDC clinical stage	**N/A**	17	(65.4)	155	1		
	B	6	(23.1)	132	0.39	(0.15, 1.01)	0.053
	C	3	(11.5)	86	0.29	(0.08, 1.03)	0.056
HIV-RNA at recruitment	**≤50 copies/ml**	4	(13.8)	236	1		
	>50 copies/ml	25	(86.2)	156	11.07	(3.77, 32.50)	<0.001
Mode of HIV acquisition	**Blood products**	1	(4.0)	7	1		
	Vertical transmission	24	(96.0)	368	0.42	(0.05, 3.62)	0.429
	Other	0	(0.0)	-	-	-	-

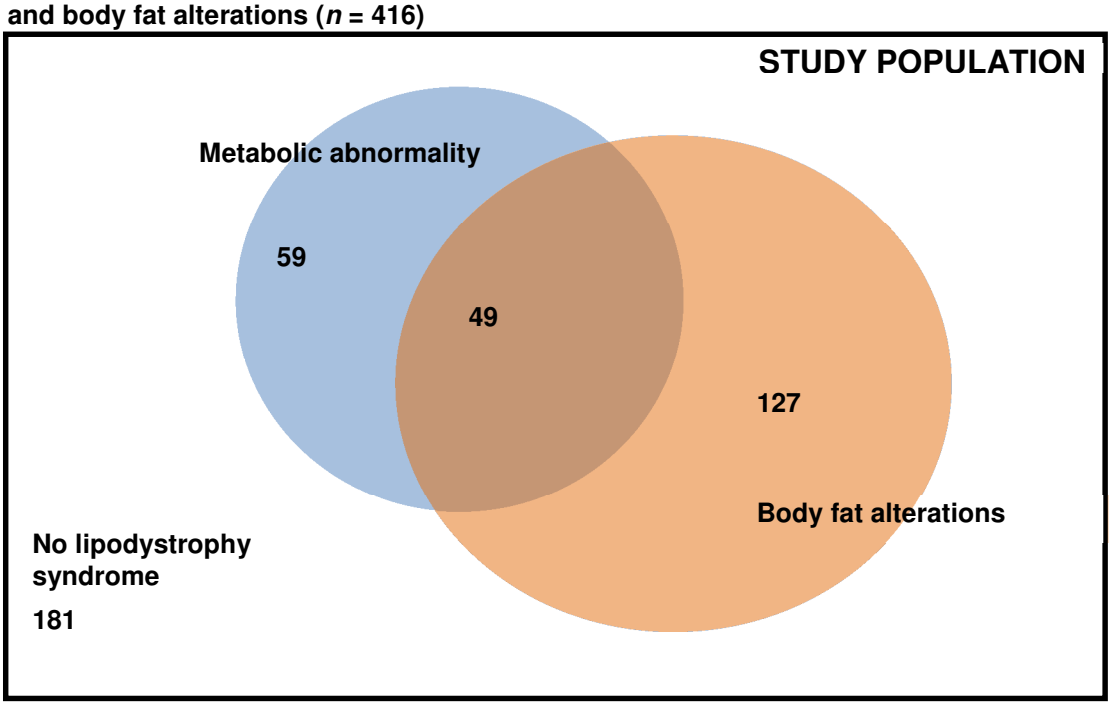
		Prevalence		Univariable analyses			
		n	**%**	***n***	**OR**	**95% CI**	***p*-value**
Other factors							
Hepatitis C co-infection	**None**	7	(96.4)	361	1		
	Co-infection	1	(3.6)	24	0.54	(0.07, 4.14)	0.551
Tanner score for puberty	**I**	11	(42.3)	123	1		
	II-IV	7	(26.9)	136	0.55	(0.21, 1.47)	0.236
	V	8	(30.8)	86	1.04	(0.40, 2.72)	0.929
BMI (kg/m^2)††		27	17.7* (15.9, 22.3)**	388	1.03	(0.92, 1.14)	0.652

CDC-defined immune status - stage 1: no immunosuppression, stage 2: moderate immunosuppression, stage 3: severe immunosuppression. CDC-defined clinical status - N + A: no symptoms/asymptomatic, B: moderate symptoms, C: severe symptoms. *median, **inter quartile range, †Odds ratio associated with age is risk associated per year of age, ††Odds ratio associated with BMI is risk associated per kg/m^2

3.8 Prevalence of lipodystrophy syndrome at recruitment

The study population size for the investigation of LS was 416: 7 subjects had missing data on metabolic abnormality, 1 had missing data on fat disorder, and 2 subjects had missing data on both. A total of 235 subjects had LS at recruitment, giving a prevalence of 56.5% (95% CI: 51.7, 61.3, *n/N* = 235/416) (Figure 3-7).

Figure 3-7: Prevalence of lipodystrophy syndrome at recruitment: metabolic abnormality and body fat alterations (*n* = 416)



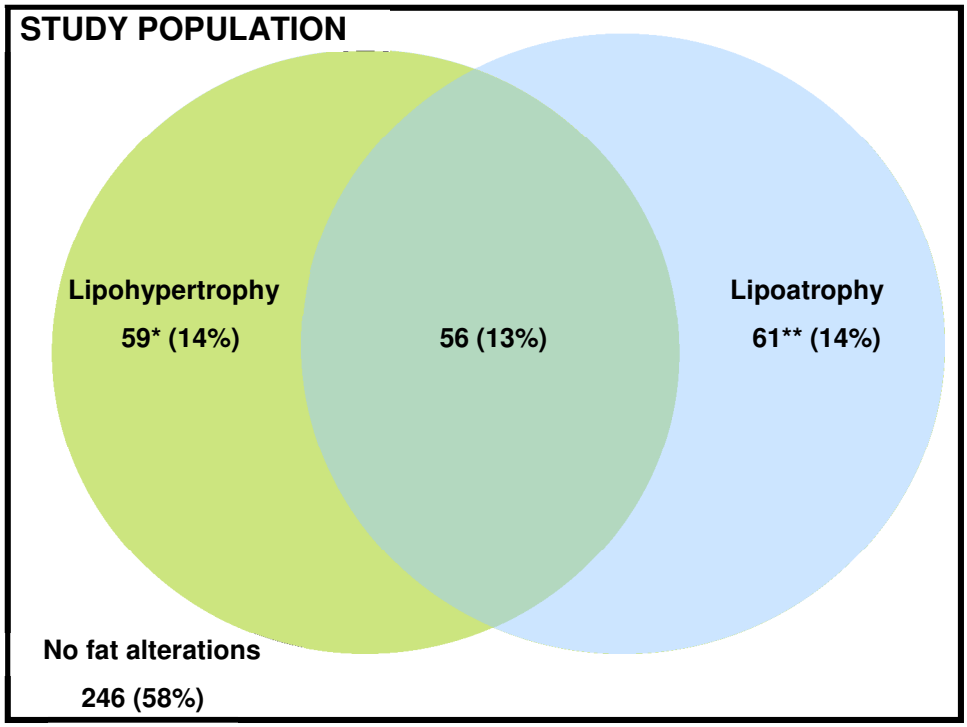
One quarter (*n/N* = 59/235) of LS cases were defined on the basis of metabolic abnormality alone, half (54.0%, *n/N* = 127/235) on the basis of body fat alterations alone, and the remainder (*n* = 49) were cases where metabolic abnormality and fat alterations occurred together. Thus, the prevalence of metabolic abnormality at recruitment was 26.0% (*n* = 108), and the prevalence of fat alterations was 41.7% (*n* = 176).

3.9 Body fat alterations at recruitment

3.9.1 Prevalence of body fat alterations

Overall, 176 subjects had body fat alterations; while a further 4 subjects had data on fat alteration missing. Thus the prevalence of body fat alterations at recruitment was 41.7% (*n/N* = 176/422). Figure 3-8 illustrates the distribution of types of body fat alterations at recruitment.

Figure 3-8: Prevalence of body fat alterations outcomes at recruitment (*n*=422).

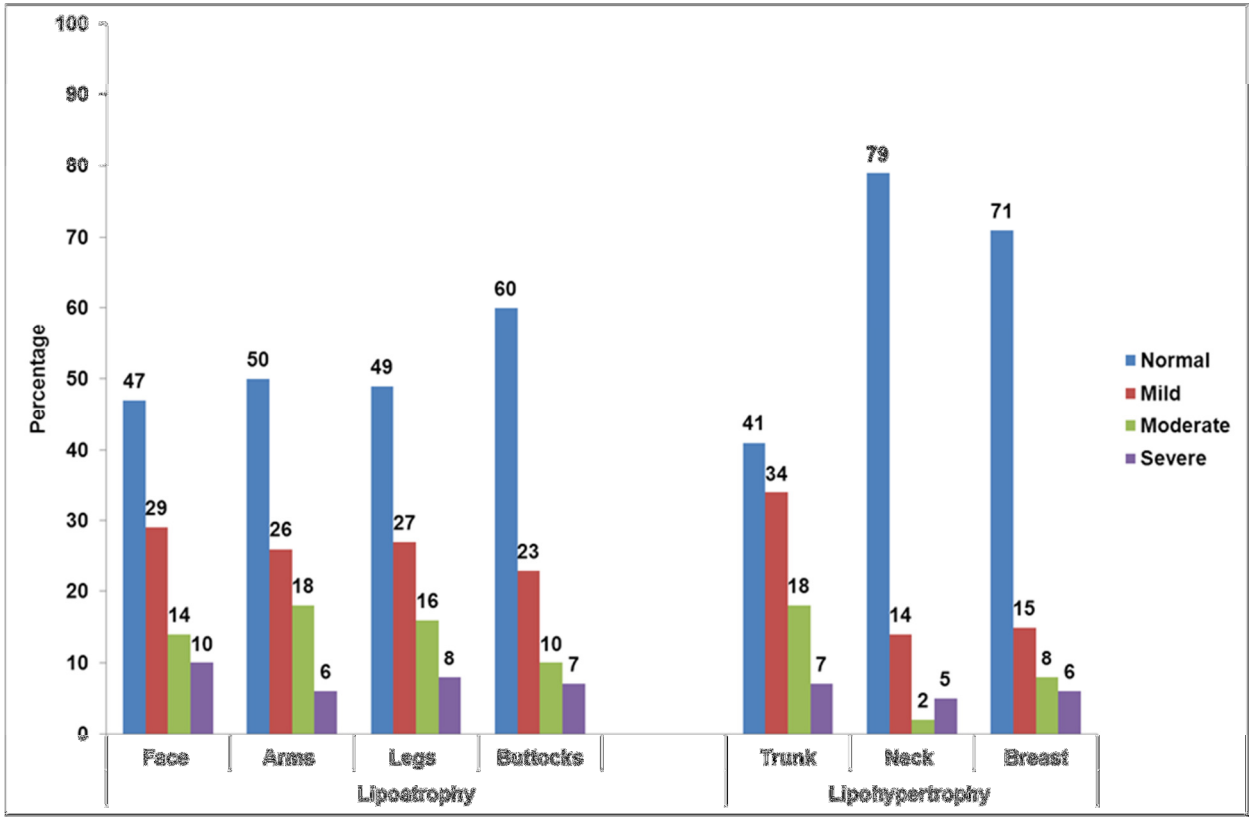


***Includes 2 subjects known to have lipohypertrophy but lipoatrophy status was unknown. **Includes 1 subject known to have lipoatrophy but lipohypertrophy status was unknown.**

The prevalence of lipohypertrophy at recruitment was 27.3% (*n/N* = 115/422). The prevalence of lipoatrophy was 27.7% (*n/N* = 117/422). Within the 176 subjects with fat alteration, one-third (*n* = 59) had lipohypertrophy alone, one third (*n* = 61) had lipoatrophy alone, and one third (*n* = 56) had both lipohypertrophy and lipoatrophy. Fat alterations was classed as being moderate or severe in 82 subjects, and severe in 28 subjects, i.e. in 46.6% and 15.9% of those 176 subjects with fat alterations respectively.

Amongst the 422 subjects with data on body fat alterations, lipoatrophy was most commonly seen in the face with a prevalence of 22.0% (*n* = 93) (Figure 3.9). The prevalence of lipoatrophy in the legs was 21.1% (*n* = 89), in the arms was 20.6% (*n* = 87), and in the buttocks was 16.1% (*n* = 68). Lipohypertrophy was most frequently seen in the trunk, with a prevalence of 24.6% (*n* = 104). The prevalence of lipohypertrophy in the neck was 8.8% (*n* = 37). Lipohypertrophy of the breast was seen 10.1% (20/199) of males and 13.7% of females (29/211): these differences were not statistically significant (χ^2 test: $p = 0.249$).

Figure 3-9: Distribution of body fat alterations by body site among the 176 subjects with alterations



Percentages are given as a proportion of the number of subjects with body fat alterations at each location: missing data: arms; 1, leg; 1, buttocks; 5, neck; 1, breasts; 1

Over half of the subjects (*n* = 95) with fat redistribution (n = 176) were affected in ≥3 locations (Table 3-6).

Table 3-6: Distribution of number of locations of body fat alterations at recruitment, by type of disorder (*n* = 176)

	Body fat alterations *n* (%)	Lipohypertrophy *n* (%)	Lipoatrophy *n* (%)
0	N/A	60(*34.5*)	57 (*33.7*)
1	52 (*29.5*)	61 (*35.0*)	25 (*14.8*)
2	29 (*16.5*)	29 (*16.7*)	12 (*7.1*)
3	26 (*14.7*)	24 (*13.8*)	22 (*13.0*)
4	32 (*18.2*)	0	53 (*31.4*)
5	13 (*7.4*)	0	0
6	12 (*6.8*)	0	0
7	12 (*6.8*)	0	0
TOTAL	176(100)	174(100)	169(100)

Missing data: lipohypertrophy; 2, and lipoatrophy; 7

Over three-quarters (*n* = 87) of the 112 subjects with lipoatrophy, and 46.5% (*n* = 53) of 114 subjects with lipohypertrophy were affected in ≥2 locations. The majority (62%) of subjects with one sign of fat alteration had mild (*n* = 25) or moderate (*n* = 6) trunk lipohypertrophy, while 22% (*n* = 11) had mild facial lipoatrophy. This may be indicative of a conservative approach to diagnosing body fat alterations in this cohort, since most cases were identified with one location of fat alterations and it could be argued that alteration is more apparent in the trunk and face.

Associations between the occurrence of fat alterations in one body location with the occurrence in another specific location was tested using χ^2 tests significant associations ($p < 0.001$) were found between fat alterations at all pairs of locations for both lipoatrophy and lipohypertrophy.

Proportions of subjects with lipoatrophy at a given body location were consistently higher in males compared to females, but these differences were not significant (Table 3-8). Similarly the proportion with lipohypertrophy at specific body locations was higher in females compared to males: these differences were significant in the trunk and in the neck ($p < 0.05$) only.

Table 3-7: Presence of fat alteration at specific body locations, stratified by sex

		MALE		FEMALE		*p*-value
		n/N	**%**	***n/N***	**%**	
LIPOATROPHY	**Face**	47/199	*24*	43/212	*20*	0.414
	Arms	46/198	*23*	39/212	*18*	0.227
	Legs	45/198	*23*	41/212	*19*	0.400
	Buttocks	33/196	*17*	31/210	*15*	0.566
LIPO-HYPERTROPHY	**Trunk**	38/199	*19*	63/212	*30*	0.012
	Neck	11/198	*6*	24/212	*11*	0.037
	Breasts	20/199	*10*	29/211	*14*	0.249

Proportions of male and female subjects with fat redistribution at specific sites compared with χ^2 test for association

Table 3-10 illustrates the age and gender stratified prevalence of fat alterations at specific body sites. There was greater prevalence of lipoatrophy in the arms, legs and buttocks in the 12-18 and 7-11 year age groups compared with the 2-6 year old age group in both males and females. However, significant differences in facial lipoatrophy by age were only seen in males.

Table 3-8: Associations between body site of fat alterations and age, stratified by sex

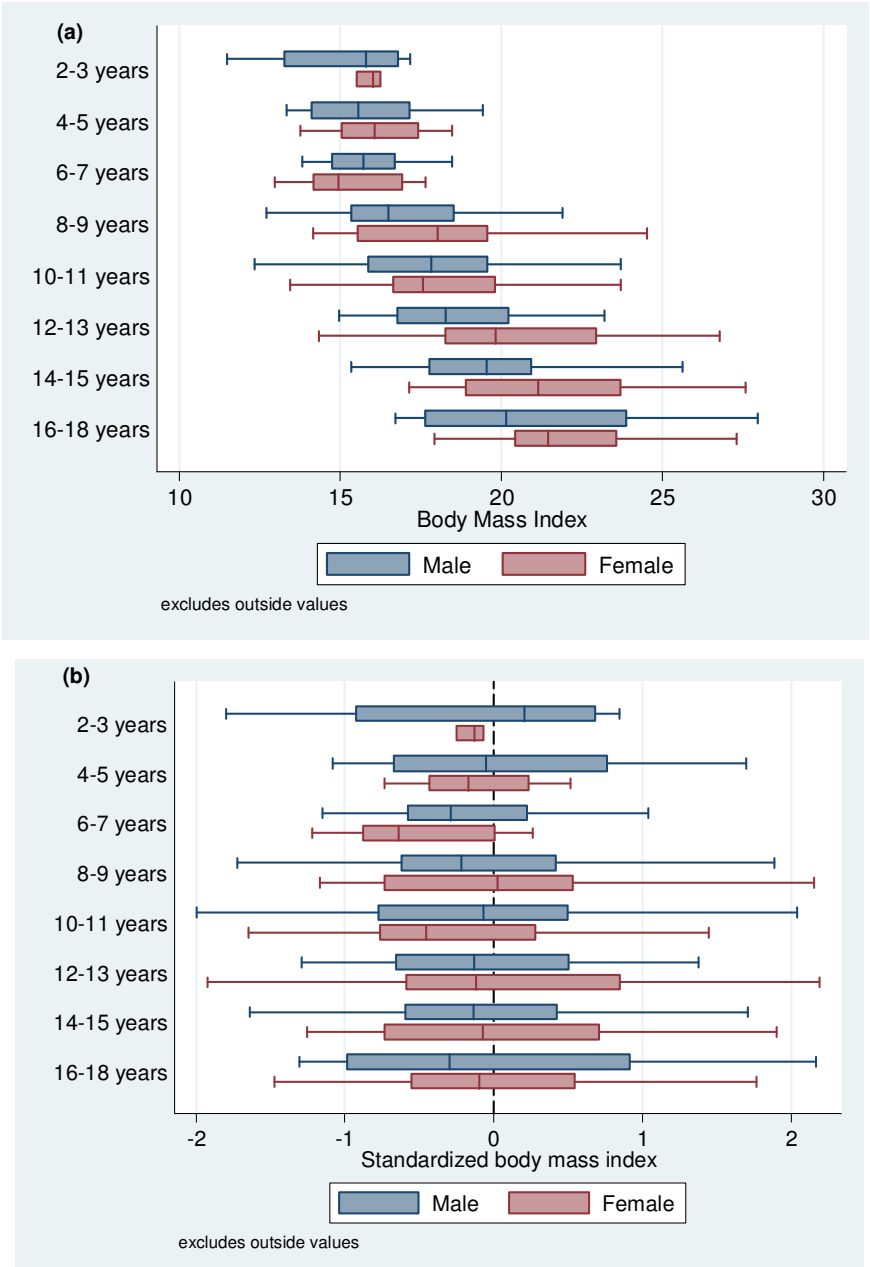
		MALE							FEMALE						
		2-6 years		7-11 years		12-18 years		*p*-value	2-6 years		7-11 years		12-18 years		*p*-value
		n/N	%	*n/N*	%	*n/N*	%		*n/N*	%	*n/N*	%	*n/N*	%	
LIPOATROPHY	**Face**	1/21	*4.8*	9/49	*18.4*	37/129	*28.7*	0.035	2/21	*9.5*	12/94	*12.8*	29/127	*22.8*	0.349
	Arms	0/21	*0*	11/49	*22.4*	35/128	*27.3*	0.023	1/21	*4.8*	8/64	*12.5*	30/127	*23.6*	0.041
	Legs	0/21	*0*	10/49	*20.4*	35/128	*27.3*	0.019	1/21	*4.8*	8/64	*12.5*	32/127	*25.2*	0.023
LIPO-HYPERTROPHY	**Buttocks**	0/21	*0*	6/47	*12.8*	27/128	*21.1*	0.039	0/21	*0*	5/62	*8.1*	26/127	*20.5*	0.010
	Trunk	2/21	*9.5*	7/49	*14.3*	29/129	*22.5*	0.230	3/21	*14.3*	19/64	*29.7*	41/127	*32.3*	0.247
	Neck	0/21	*0*	2/49	*4.1*	9/128	*7.0*	0.374	1/21	*4.8*	7/64	*10.9*	16/127	*12.6*	0.572
	Breast	0/21	*0*	3/49	*6.1*	17/129	*13.2*	0.101	0/21	*0*	7/64	*10.9*	22/126	*17.5*	0.073

Proportions with presence of body fat alterations by age compared using χ^2 test for association in male and in female subjects

3.9.2 Body mass index

Figure 3-10 illustrates median BMI and standardized BMI across age groups in both males and females. Data were available to calculate BMI for 98% (*n* = 418) of the study population, and standardized BMI for 96% (*n* = 408), i.e. data were missing for 8 (BMI) and 18 (BMI and/or gender) subjects respectively.

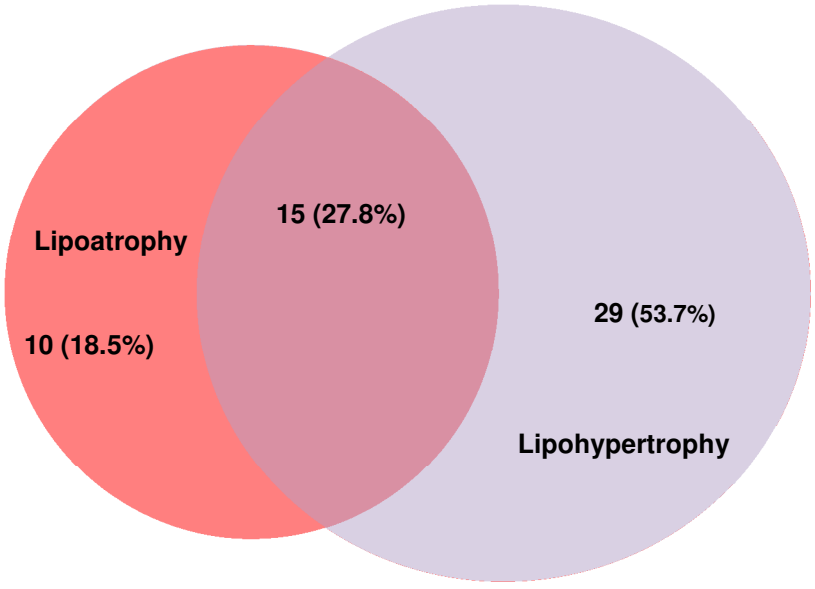
Figure 3-10: BMI across age groups: (a) median BMI and (b) median standardized BMI



Boxes demark 25th and 75th percentiles (2nd and 3rd quartile) and lines indicate adjacent values (most extreme values within 1.5 interquartile range of the nearest quartile) of the distribution within each age band. Values outside adjacent values excluded for clarity. *n* = 418 for BMI, and *n* = 408 for standardized BMI. Comparison of median standardized body mass index between males and females: *p* > 0.05 for all groups.

Median BMI increased with age, as would be expected. Moreover a slower increase in BMI was seen in younger age groups compared to older age groups. This reflect so-called adiposity rebound where BMI increases in the first year of life but then subsequently decreases before increasing again from the age of 5 years[386], although increased BMI was seen in this population at aged 8 years onwards. Using the CDC sex-specific BMI-for-age definitions, 36 subjects were overweight, 43 were at risk of being overweight and 18 subjects were underweight. Of the overweight, 63.9% (*n/N* = 23/36) had body fat alterations: this percentage was 55.8% (*n/N* = 24/43) for those at risk of being overweight and 38.9% (*n/N* = 7/18) amongst those who were underweight. Figure 3-11 illustrates the specific fat disorder in these 54 subjects who were in the CDC-defined unhealthy BMI categories and had diagnosis of specific body fat alteration: more than half had lipohypertrophy alone, while a quarter had both lipohypertrophy and lipoatrophy.

Figure 3-11: Body fat characteristics of subjects with body fat alterations and who were CDC-defined underweight, potentially overweight or overweight (*n* = 54)



Two subjects had a BMI greater than 30, the definition of obesity in adults [387]. Both of these subjects were females aged over 14 years and both had body fat abnormality: the first with moderate symptoms of combined lipoatrophy and lipohypertrophy, while the second had lipohypertrophy in 3 locations including severe symptoms in the trunk.

The fractional polynomial model for BMI with recruitment age as an explanatory variable in subjects with LS was different to the model in subjects without LS (Figure 3-12); this was true

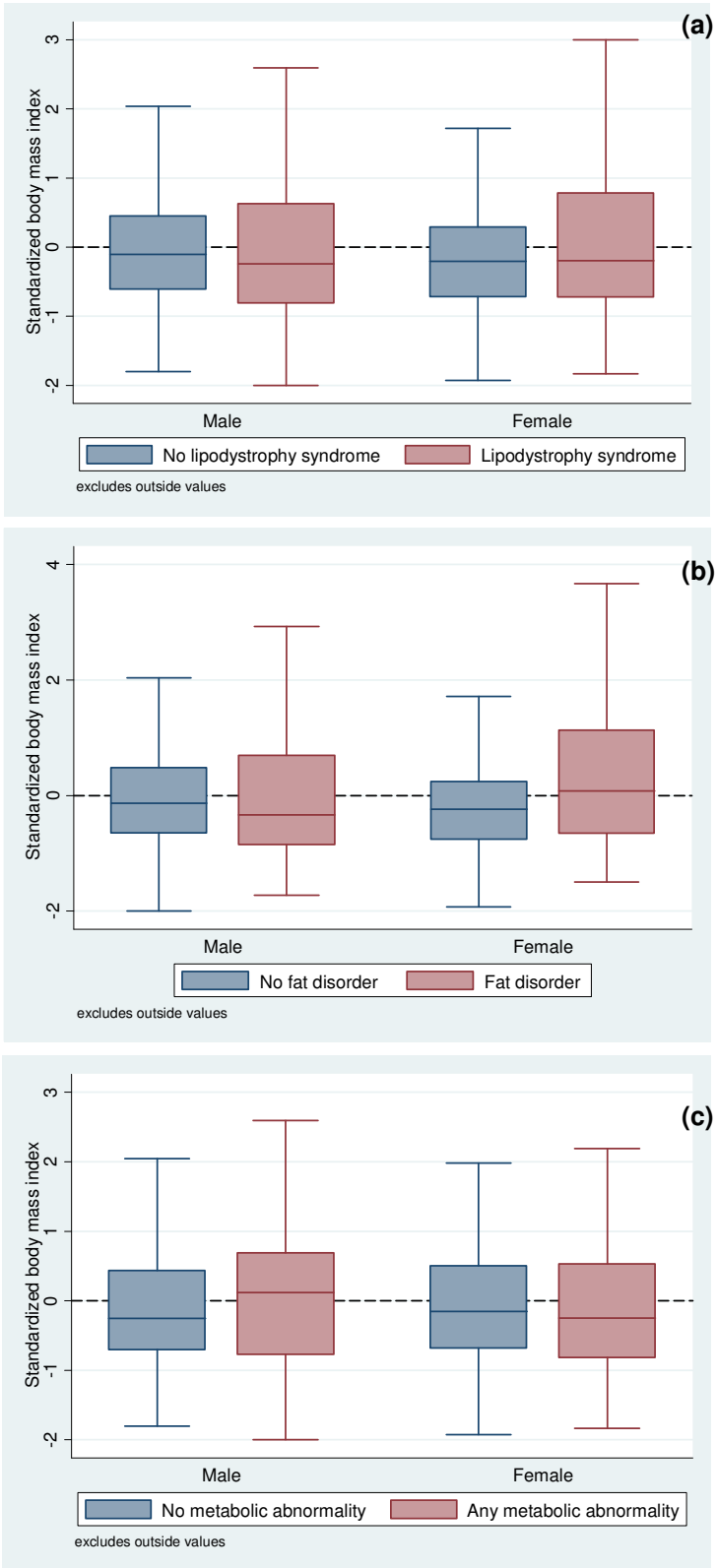
for models for males and those for females, i.e. while the order of the Box-Tidwell transformations in both models was 2, age was raised to different powers within each model, and estimates of accompanying coefficients also being different. This may be indicative of differences in the relationship between age at recruitment and BMI which may be associated with LS, with further differences due to sex. Smooth curves generated from the fractional polynomial models also indicated that subjects who were older at recruitment had higher BMI compared to younger subjects. For both sexes, examination of scatterplots suggested a greater heterogeneity in the individual age and BMI values in subjects with LS compared to those without (Figure C-2 in Appendix C). Furthermore, the smooth curve for males with LS suggests that adipose rebound may occur later in these subjects: however, estimates of parameter only reached statistical significance in females (Appendix C, Table C-5). For both sexes estimates of BMI at given age tended to be slightly higher in subjects with LS.

Further visual inspection of curves to subjects without LS did not indicate that the relationship between BMI and age was different during puberty (i.e. between the ages of 9 and 15 years in females, and between 11 and 16 years in males), compared to either before or following puberty. However, there was evidence that the relationship between BMI and age did change during puberty in subjects with LS, as seen by the fluctuations in these curves.

Similarly, the fractional polynomial models were different between subjects with fat disorder compared to those without in both males and females (Appendix C: Figure C-2 and Table C-6): in these models parameters reached significance in both male and female models. However, there was no significant difference in median values for standardized BMI between subjects with/without lipodystrophy syndrome, body fat abnormality, or metabolic abnormality (Figure 3-12) amongst either males or females. Furthermore, there was no difference in median values between males and females, nor any median difference between subjects with each outcome, compared to subjects without the outcome. The aim of the fractional polynomial models is to indirectly compare those with/without LS by assessing the differences in structure of the models thus characterizing the changes in BMI with age. However, the median comparisons investigate difference in overall BMI. This explains why the fractional polynomial models were seen to be different between groups, while the tests for differences in medians were statistically non-significant.

Thus while there is evidence that the relationship between age at recruitment and BMI varies in subjects with LS, there is no significant difference in median standardized values of BMI when comparing those with LS to those without.

Figure 3-12: Comparison of median standardized BMI, stratified by sex, between subjects with/without (a) lipodystrophy syndrome, (b) fat alterations, and (c) metabolic abnormality

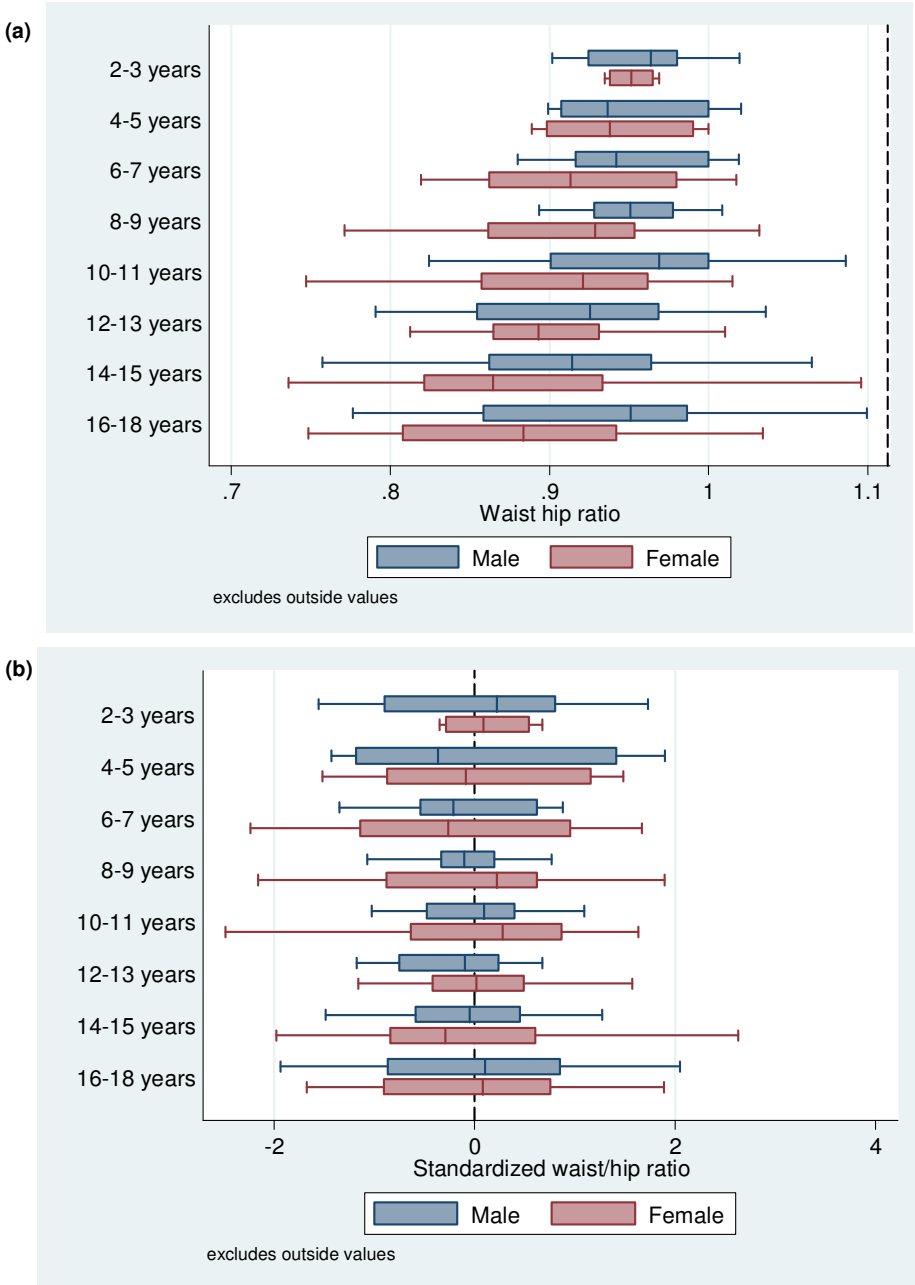


Boxes demark 25th and 75th percentiles (2nd and 3rd quartile) and lines indicate adjacent values (most extreme values within 1.5 interquartile range of the nearest quartile) of the distribution within each age band. Values outside adjacent values excluded for clarity. Comparison of median standardized BMI between subjects with outcome and without outcome: $p \geq 0.190$.

3.9.3 Waist/hip ratio

Waist/hip ratio was available for over almost three-quarters of the population (*n* = 317), while standardized estimates were available for over 70% (*n* = 310). Male median waist/hip ratio was greater than female at a given age, but these differences were not statistically significant (Figure 3-13). Furthermore, a non-significant decrease in median waist/hip ratio was seen with increasing age.

Figure 3-13: Waist/hip ratio across age groups: (a) median waist/hip ratio, and (b) median standardized waist/hip ratio



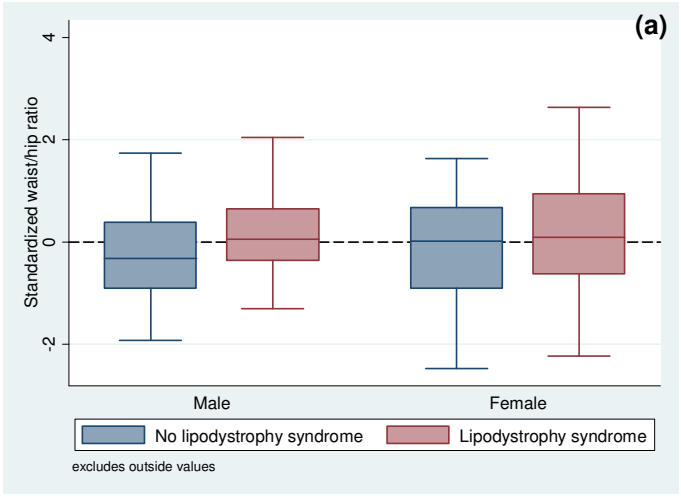
Boxes demark 25th and 75th percentiles (2nd and 3rd quartile) and lines indicate adjacent values (most extreme values within 1.5 interquartile range of the nearest quartile) of the distribution within each age band. Values outside adjacent values excluded for clarity. *n* = 317 for waist/hip ratio, and *n* = 310 for standardized waist/hip ratio. Comparison of median standardized waist/hip ratio between males and females: *p* > 0.05 for all groups.

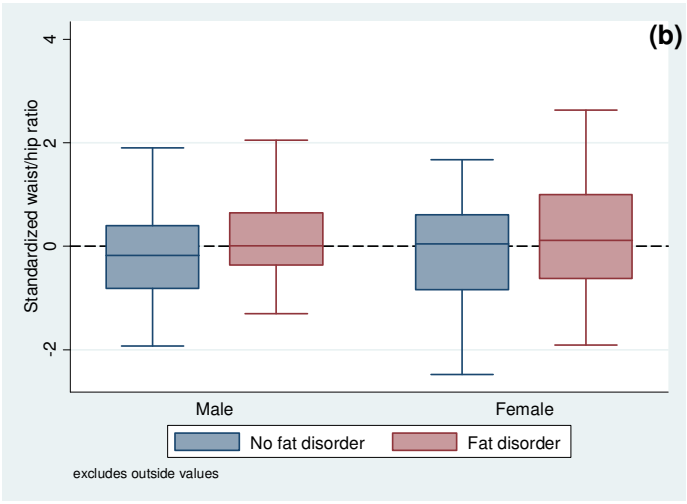
In males, the fractional polynomial model for waist/hip ratio by age at recruitment had a similar structure in both the model estimated for subjects with lipodystrophy and the model estimated for subjects without, i.e. within both models age was raised to the same powers although coefficients did not have the same value (Figure 3-15). However, the fractional polynomial model for females with LS differed in structure compared to the model for females without LS. Male and female models were also different. Thus, the relationship between age at recruitment and waist/hip ratio between males and females may be different, and furthermore the relationship may be different in females with LS compared to females without LS. Moreover, LS did not seem to affect the pattern of age at recruitment and waist/hip ratio in males. However, coefficients of age were not statistically significant in any model (Appendix C: Table C-7).

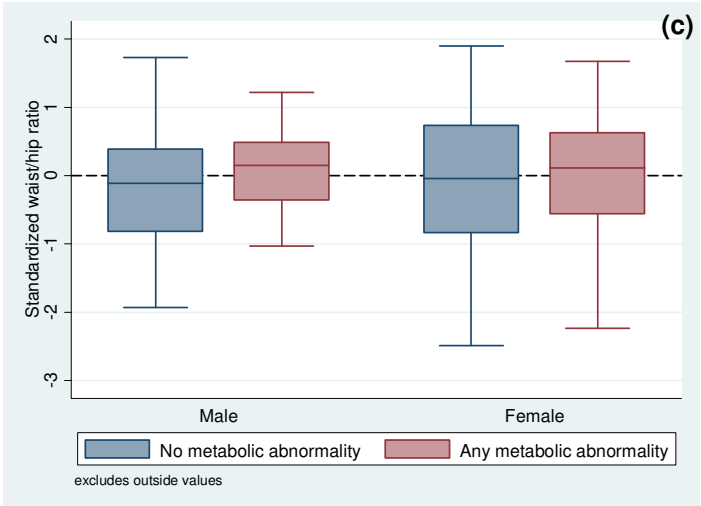
As illustrated in (Figure 3-14), standardized waist/hip ratio was comparable between subjects with LS compared to those without, those with fat disorder compared to those without, and those with metabolic abnormality compared to those without. Furthermore there were no differences seen between sexes for any outcome.

Thus, while fractional polynomial modelling suggests that LS affects the relationship between age at recruitment and waist/hip ratio in girls, no significant difference was seen in standardized waist/hip ratio between subjects with/without LS outcomes in either males or females.

Figure 3-14: Comparison of median standardized waist/hip ratio, stratified by sex, between subjects with/without (a) lipodystrophy syndrome, (b) fat alterations, and (c) metabolic abnormality







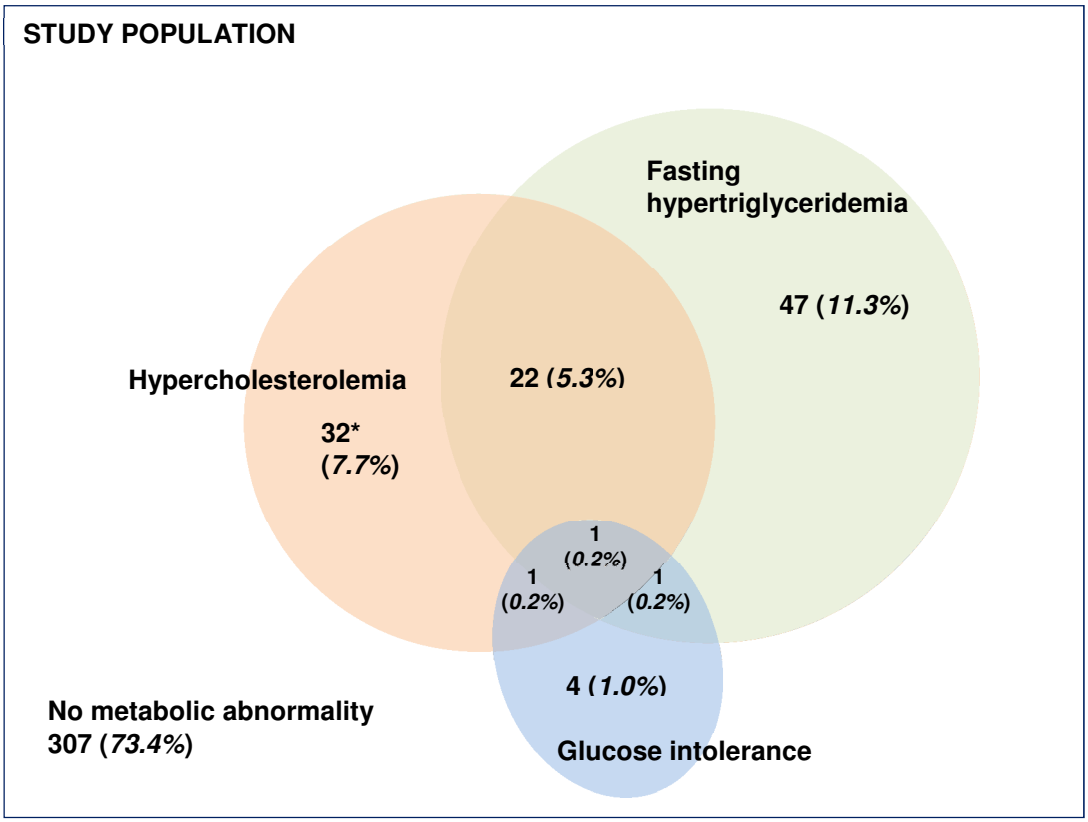
Boxes demark 25th and 75th percentiles (2nd and 3rd quartile) and lines indicate adjacent values (most extreme values within 1.5 interquartile range of the nearest quartile) of the distribution within each age band. Values outside adjacent values excluded for clarity. Comparison of median standardized waist/hip ratio between subjects with outcome and without outcome: $p \geq 0.157$.

3.10 Metabolic abnormality at recruitment

3.10.1 Prevalence of metabolic abnormality

The prevalence of metabolic abnormality at recruitment was 26.0% (*n* = 108) (Figure 3-7. Prevalence of fasting hypertriglyceridemia was 17.0% (*n* = 71), of hypercholesterolemia was 13.4% (*n* = 56), and of glucose intolerance was 1.6% (*n* = 7).

Figure 3-15: Frequencies of metabolic abnormality outcomes of hypercholesterolemia, fasting hypertriglyceridemia and glucose intolerance (*n* = 419):



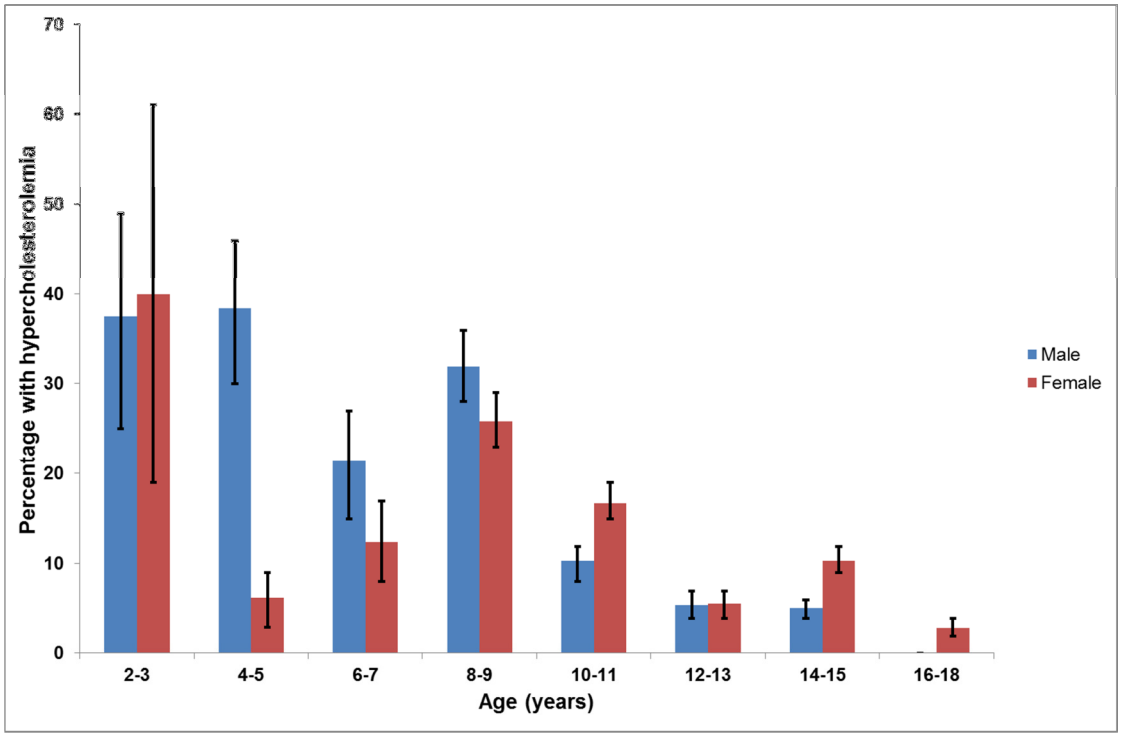
***Includes 1 case where hypercholesterolemia and fasting hypertriglyceridemia status was known, but glucose tolerance was not known, and 1 case where hypercholesterolemia and glucose intolerance was not known while fasting hypertriglyceridemia status was not known.**

Less than half (43.5%, *n* = 47) of the 108 subjects with metabolic abnormality were defined by fasting hypertriglyceridemia alone, while 29.6% (*n* = 32) were defined by hypercholesterolemia alone, and 3.7% (*n* = 4) by glucose intolerance alone (Figure 3-13). The remaining 29 subjects with metabolic abnormality had mixed phenotype: 20.4% (*n/N* = 22/108) had both hypercholesterolemia and fasting hypertriglyceridemia, 1 had combined glucose intolerance and fasting hypertriglyceridemia, 1 had both glucose intolerance and hypertriglyceridemia, and, 1 had glucose intolerance, hypercholesterolemia and fasting hypertriglyceridemia.

3.10.2 Cholesterol

Overall prevalence of hypercholesterolemia was 13.4% (*n/N* = 56/423), with proportions of subjects with hypercholesterolemia higher in younger age groups compared with older age groups (Figure 3-16). There was a significant association between increasing age and decreasing cholesterol concentration ($p < 0.001$). In three subjects, data necessary to categorize the subject as a hypercholesterolemia case were missing.

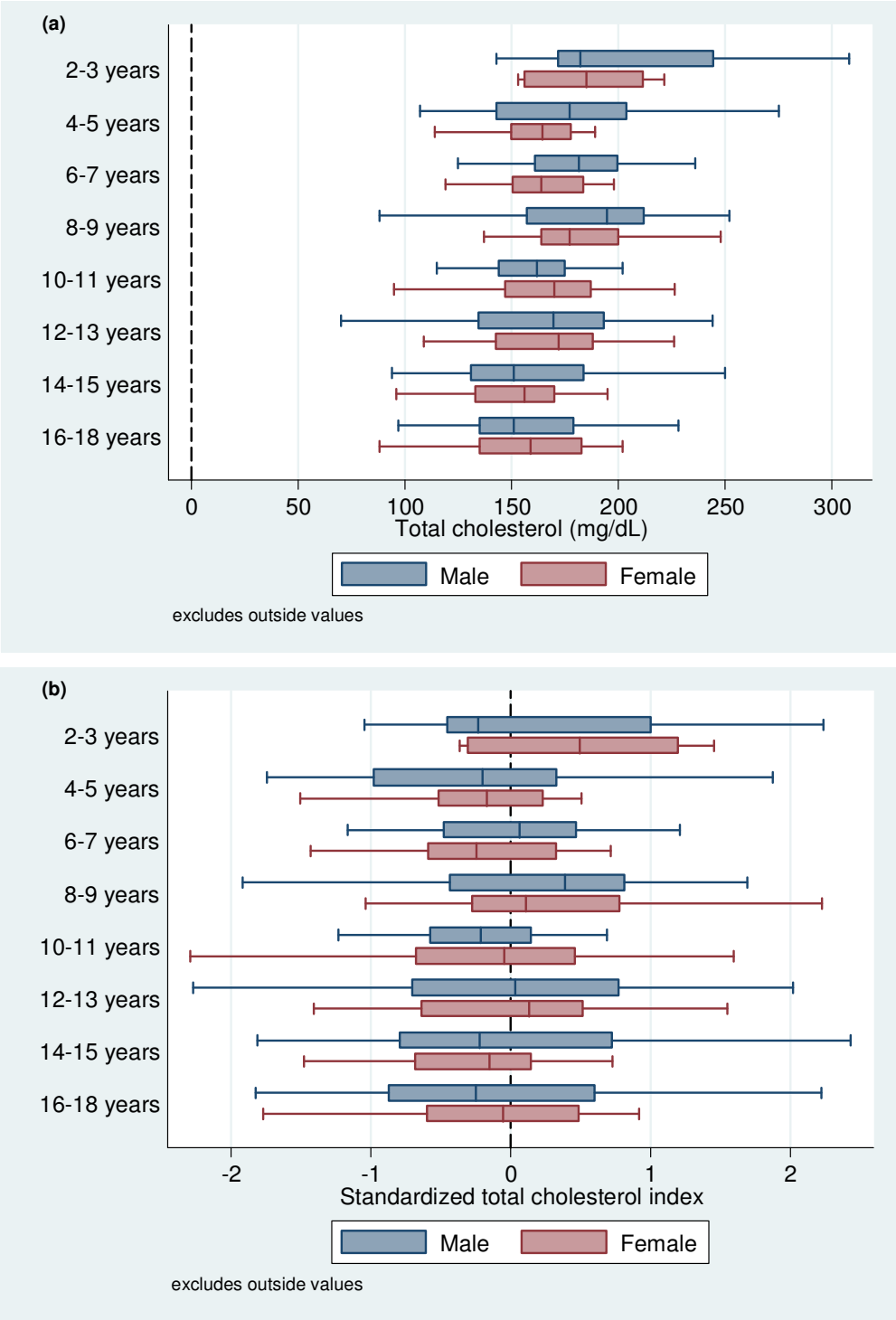
Figure 3-16: Distribution of hypercholesterolemia by age and gender (*n* = 412)



2-3 years: male *n*=8, female *n* =16, 4-5 years: males *n*=13, females *n*=16, 6-7 years: males *n*=14, females *n*=16, 8-9 years: males *n*=25, females *n*=31, 10-11 years: males *n*=29, females *n*=36, 12-13 years: males *n*=37, females *n*=36, 14-15 years: males *n*=39, females *n*=39, and 16-18: males *n*=34, females *n*=34.

Measures of cholesterol were missing for 7 subjects and data required for calculation of age and gender standardized cholesterol was missing for a further 11 subjects: thus population-standardized cholesterol could be estimated for 95.8% (*n/N* = 408/426) of subjects. Median cholesterol levels were non-significantly higher in males compared to females in the 2-3, 4-5, 6-7 and 8-9 age groups, with a decrease in levels in these groups with ascending age (Figure 3-17). However, median standardized cholesterol had no discernible patterns by gender or age group.

Figure 3-17: Total cholesterol across age groups: (a) median total cholesterol, and (b) median standardized total cholesterol



Boxes demark 25th and 75th percentiles (2nd and 3rd quartile) and lines indicate adjacent values (most extreme values within 1.5 interquartile range of the nearest quartile) of the distribution within each age band. Values outside adjacent values excluded for clarity. *n* = 419 for cholesterol, and *n* = 408 for standardized cholesterol Comparison of median standardized total-cholesterol between males and females: $p > 0.05$ for all groups.

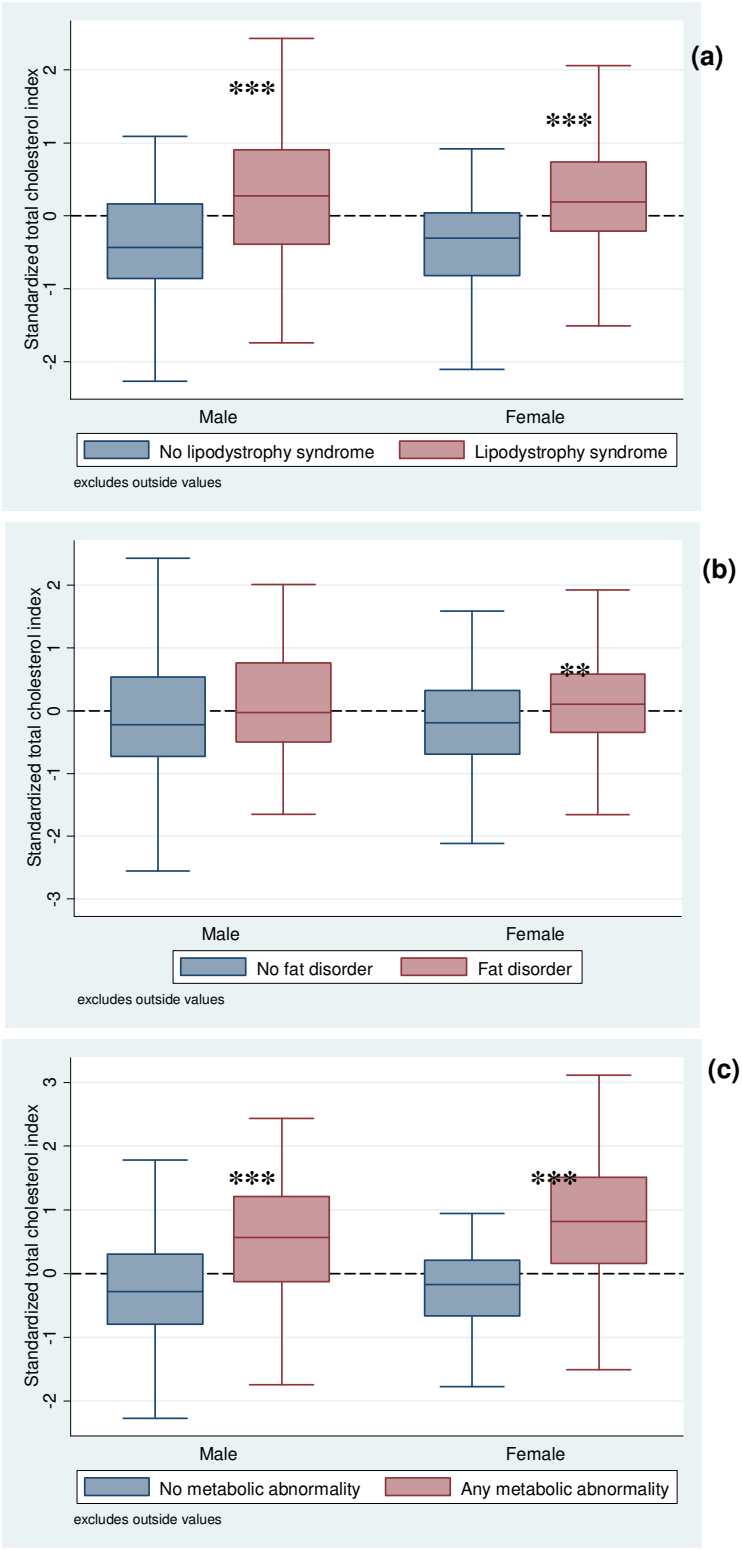
Examination of scatterplots of age at recruitment with total serum cholesterol, suggests heterogeneity which may be more apparent in subjects with LS (Figure 3-18). However, plots using fractional polynomial modelling suggest that total serum cholesterol may vary little across subjects of increasing age at recruitment. Indeed the fractional polynomial models for males with LS, males without LS and females with LS all had a similar structure with age raised to the same powers in these three models (Appendix C: Table C-9). However, the fractional polynomial model for females without LS was of a different form. Statistical significance varied for coefficients across models.

These models may suggest that LS does not influence the association between age at recruitment and serum cholesterol in males. The models in males had the same structure but the estimated coefficients were of different value. Thus, although the slope between models is not different, the intercept is different: for a given age, total cholesterol in males with LS is higher than in males without LS. Since the regression function is different for lipodystrophy females compared to those without lipodystrophy, LS may be an influential factor on total serum cholesterol concentration in females.

Regarding subjects with/without metabolic abnormality, all models had different structures (Appendix C: Table C-10), which suggests that metabolic abnormality has an effect on the relationship between age and total cholesterol in both males and females

As would be expected, given hypercholesterolemia is a component of the definitions of metabolic abnormality and LS, total cholesterol levels were seen to be increased in subjects with LS compared to those without, and in subjects with metabolic abnormality compared to those without (Figure 3-21). Furthermore, standardized total cholesterol was similar in males and females across outcomes. Although subjects with fat disorder had increased standardized total cholesterol compared to subjects without fat disorder, but this difference was only significant in females ($p = 0.008$).

Figure 3-18: Comparison of median standardized total cholesterol, stratified by sex, between subjects with/without (a) lipodystrophy syndrome, (b) fat alterations, and (c) metabolic abnormality



Boxes demark 25th and 75th percentiles (2nd and 3rd quartile) and lines indicate adjacent values (most extreme values within 1.5 interquartile range of the nearest quartile) of the distribution within each age band. Values outside adjacent values excluded for clarity. Comparison of median standardized total cholesterol ratio between subjects with outcome and without outcome: *$p < 0.01$, **$p < 0.01$, ***$p < 0.001$.

Low density lipoprotein (LDL) cholesterol

LDL-cholesterol data was available for 81.9% (349/426) of subjects (77 missing). Age- and gender- standardized estimates for LDL cholesterol could be calculated for 80.0% (341/426). Male subjects had median LDL-cholesterol levels similar or greater than median levels seen in females (Appendix C: Figure C-6).

As with total cholesterol, there was great heterogeneity between measures of age at recruitment and serum levels of LDL cholesterol (Appendix C: Figure C-8). However, differences in degree of heterogeneity between subjects with LS and those without were not as obvious. The structure of the fractional polynomial models investigating the cross sectional relationship between age and LDL-cholesterol in males with/without LS both had age raised to the same powers: this may imply that LS does not affect the relationship between age and LDL cholesterol (Appendix C: Table C-11). However, the models in subjects with/without LS did not have the same structure in females, indicating that LS may have an impact on this relationship: this is not surprising given that LS was seen to be influential on total cholesterol levels in females in the previous section. While estimates of the intercepts in all four models were statistically significant, estimates of all coefficients were not. The regression models suggest that females with LS had higher values of LDL cholesterol at a given age at recruitment compared to females without LS. Males with LS had similar values of LDL-cholesterol at a given age as males without LS.

Subjects with LS had significantly higher levels of standardized LDL-cholesterol compared to subjects without LS (Appendix C: Figure C-9). While subjects with fat disorder had higher LDL-cholesterol compared to subjects without fat disorder, these differences were not statistically significant.

Thus, while there was evidence that LS had an effect on the relationship between age at recruitment and LDL-cholesterol, these results suggest that these differences may only be apparent in females.

Non-high density lipoprotein (non-HDL) cholesterol

Data on non-HDL cholesterol was estimated in 84.5% (360/426) of the study population, and standardized non-HDL cholesterol was calculated for 82.4% (351/426). Median non-HDL cholesterol in males was higher in males than in females in all age groups except the 10-11 year olds (Appendix C: Table C-11).

Fractional polynomial models in males showed that levels of non-HDL-cholesterol oscillated with increasing age of recruitment: this was especially true of males without LS (Appendix C: Figure C-14 and Table C-13). There were also lower levels in older children compared to younger children in subjects with LS. In contrast, the fractional polynomial models for females with/without LS were relatively flat, with older children having similar levels of non-HDL-cholesterol as younger children at recruitment. This is reflected in the fact that all models

differed in structure. While the regression functions suggested that males with LS had higher values of non-HDL-cholesterol at a given age compared to males without LS, this difference was more visibly apparent for females. Indeed the structure of the fractional polynomial models was the same between males with/without LS, but differed between females with LS and those without, although the majority of coefficients were statistically non-significant. As with total and LDL cholesterol, this suggests that LS has an influence on serum concentrations of non-HDL cholesterol in females but not in males.

Standardized measures of non-HDL cholesterol were significantly higher in subjects with LS compared to subjects without, and in subjects with metabolic abnormality compared to those without, in both males and females (Appendix C: Figure C-15). Despite this being expected (as hypercholesterolemia is part of the definition of both LS and metabolic abnormality used in this study), the differences were of a greater magnitude than seen for standardized measures of LDL-cholesterol (also part of the definition of LS) between subjects with/without outcome. While median standardized non-HDL-cholesterol was higher in subjects with fat disorder compared to those without, this was only significant in females.

High density lipoprotein (HDL) cholesterol

HDL-cholesterol was missing in 66 subjects, and thus was available for 84.5% of subjects. Sufficient data were available to estimate gender- and age- standardized values for 82% (351/426) of the study population. HDL-cholesterol was only higher in females compared with males in the 10-11 year olds: in all other age groups, female levels were lower than those in males (Appendix C: Figure C-16). The only significant sex difference in age and gender standardized HDL-cholesterol was seen among 6-7 year olds ($p = 0.019$).

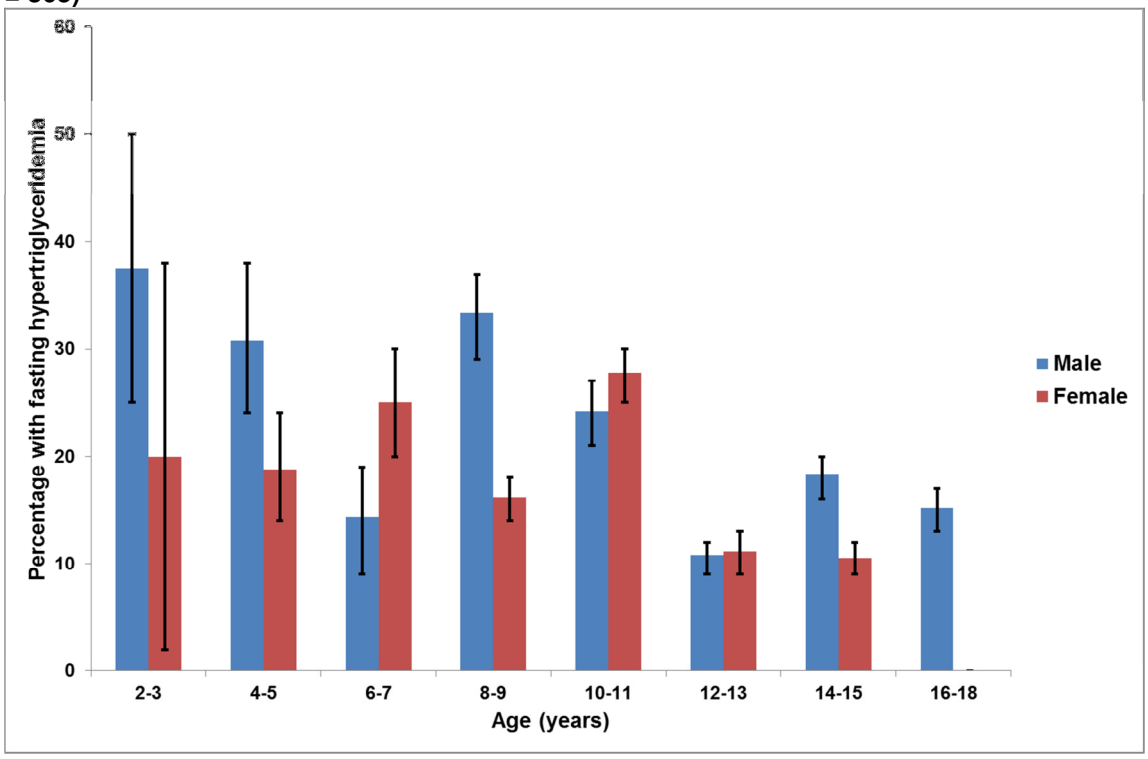
Figure C-18 in Appendix C illustrates scatter plots with estimated fractional polynomial curves using age at recruitment as the explanatory factory and serum HDL-cholesterol as the response variable: plots are shown for males with/without LS, and females with/without LS. All plots showed heterogeneity in the observed values of age and HDL-cholesterol: there was no evidence of differences in the degree of heterogeneity between subjects with/without LS The regression functions for males were similar to those for females (Appendix C: Table C-15): in the former peaks occurred at about 7 years and 12 years, while in the latter a peak occurred before 10 years (although the timing and gradient associated with this peak was different between subjects with/without LS). Furthermore, subjects with/without LS in both sexes had similar values for HDL-cholesterol at any given age. The structure of the fractional polynomial models for males were the same (although values of coefficients were different), indicating that LS may not influence the relationship between age and HDL cholesterol. In contrast, the model for females without LS had a different structure to the model for females without LS, indicating LS may influence the relationship. No coefficients were statistically significant in any model. Thus fractional polynomial models have consistently suggested that LS may influence serum concentrations of specific cholesterols in females, but not in males.

Standardized HDL was comparable between subjects with/without LS, with/without metabolic abnormality, and with/without fat disorder in females (Appendix C: Figure C-20). In contrast, male subjects without LS, metabolic abnormality or fat disorder had higher levels of HDL-cholesterol compared to male subjects without these disorders: this difference was only significant between subjects with/without metabolic abnormality.

3.10.3 Fasting triglyceride

Data on fasting hypertriglyceridemia were available for all but 8 subjects. Almost one in five subjects had age and gender-defined fasting hypertriglyceridemia (71/418). Increasing age was significantly associated with decreases in fasting hypertriglyceridemia (p = 0.003), as illustrated in Figure 3-19). However, while this relationship remained significant when the population was restricted to females (p = 0.008) it was non-significant in males (p = 0.087).

Figure 3-19: Percentage distribution of fasting hypertriglyceridemia by age and gender (*n* = 305)



2-3 years: male *n*=4, female *n* =5, 4-5 years: males *n* =8, females *n* =11, 6-7 years: males *n* =9, females *n* =12, 8-9 years: males *n* =17, females *n* =23, 10-11 years: males *n* =24, females *n* =30, 12-13 years: males *n* =25, females *n* =26, 14-15 years: males *n* =30, females *n* =33, and 16-18: males *n* =22, females *n* =26.

In addition to the 8 subjects with missing data on fasting hypertriglyceridemia status, there were 107 subjects reported as not having fasting hypertriglyceridemia. i.e. missing specific data on whether available triglyceride data had been collected as fasting or non-fasting. Thus, almost three quarters (311/426) of the study population had complete data on fasting triglyceride measures, and age and gender standardized estimates were calculated for 73% (309/426).

Males had higher fasting triglyceride levels in all age groups except 12-13 year olds (Figure 3-20). However, there was no significant difference in standardized fasting triglyceride between males and females in any age group ($p > 0.05$).

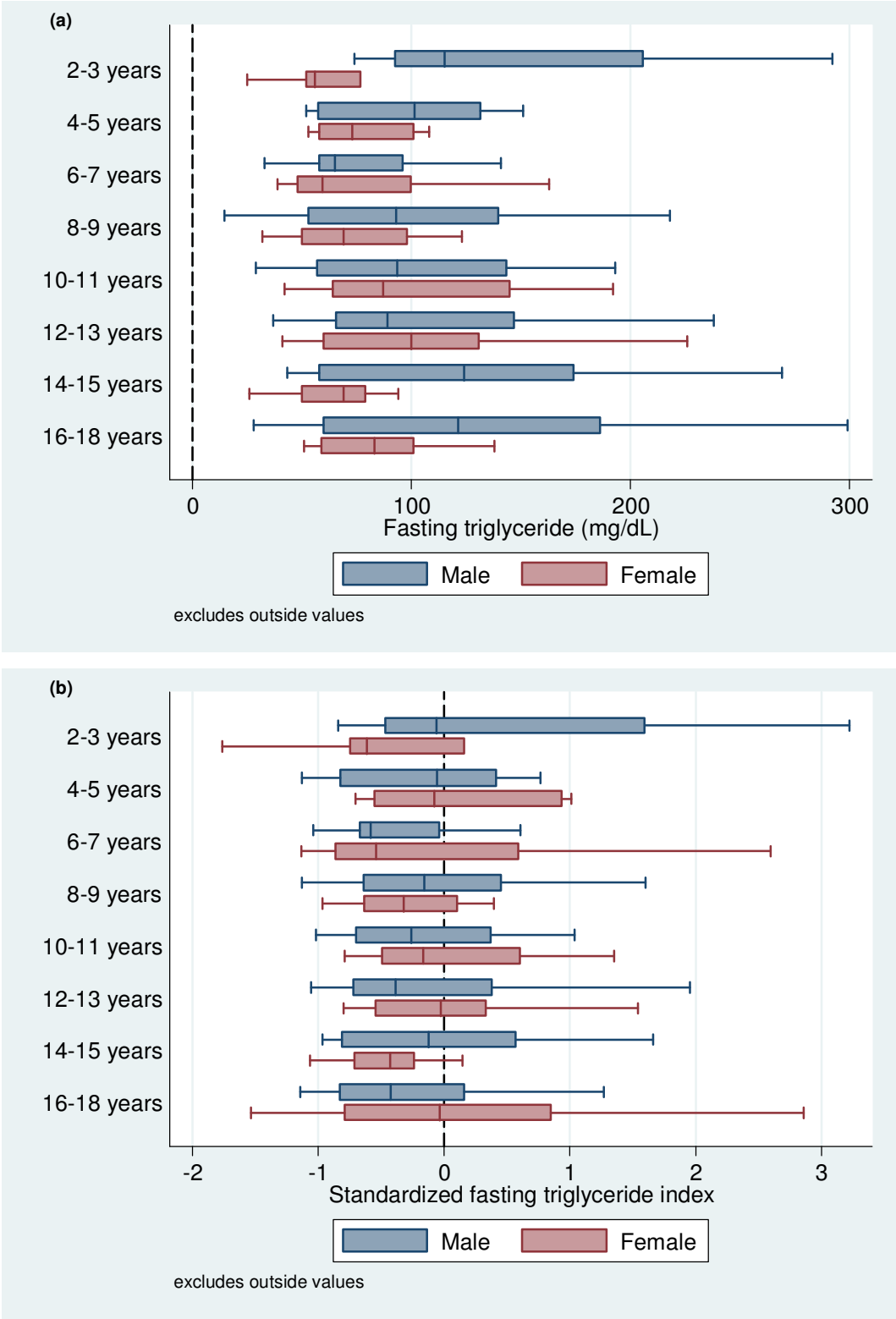
Greater heterogeneity in the observed values of fasting triglyceride (with increasing age at recruitment) was seen amongst subjects with LS compared to those without (Figure 3-24). Fractional polynomial models demonstrate a distinct pattern in males and in females. In males, subjects who are older at the time of recruitment had higher levels of fasting triglyceride than younger subjects. Furthermore, levels increase till age 12-13 years where levels briefly plateau before increasing again from age 15 years. However, in females, levels remain constant across ages with a brief increase and subsequent fall in subjects aged approximately 4 years. Across sexes, subjects with LS show exaggerated versions of these patterns compared to subjects without LS, and furthermore, levels of fasting triglyceride tend to be higher for any given age of recruitment.

In contrast to the fractional polynomial models for cholesterol, the structure of the models for subjects with LS and those without were similar for females, i.e. the explanatory variable of age at recruitment was raised to the same powers in the two models (Appendix 3: Table C-17). However, although all coefficients were statistically significant in the model of subjects without LS, no coefficients were significant in the model for subjects with LS. The structure of the model for males with LS was different to that for males without LS. This may indicate LS influencing the relationship between age at recruitment and fasting triglyceride in males.

Subjects with LS and those with metabolic abnormality had significantly higher median standardized fasting triglyceride than those without (Figure 3-21), as expected since hypertriglyceridemia is a constituent part of these definitions. Although subjects with body fat disorder had increased median standardized fasting triglyceride compared to those with no fat disorder, these differences were not statistically significant.

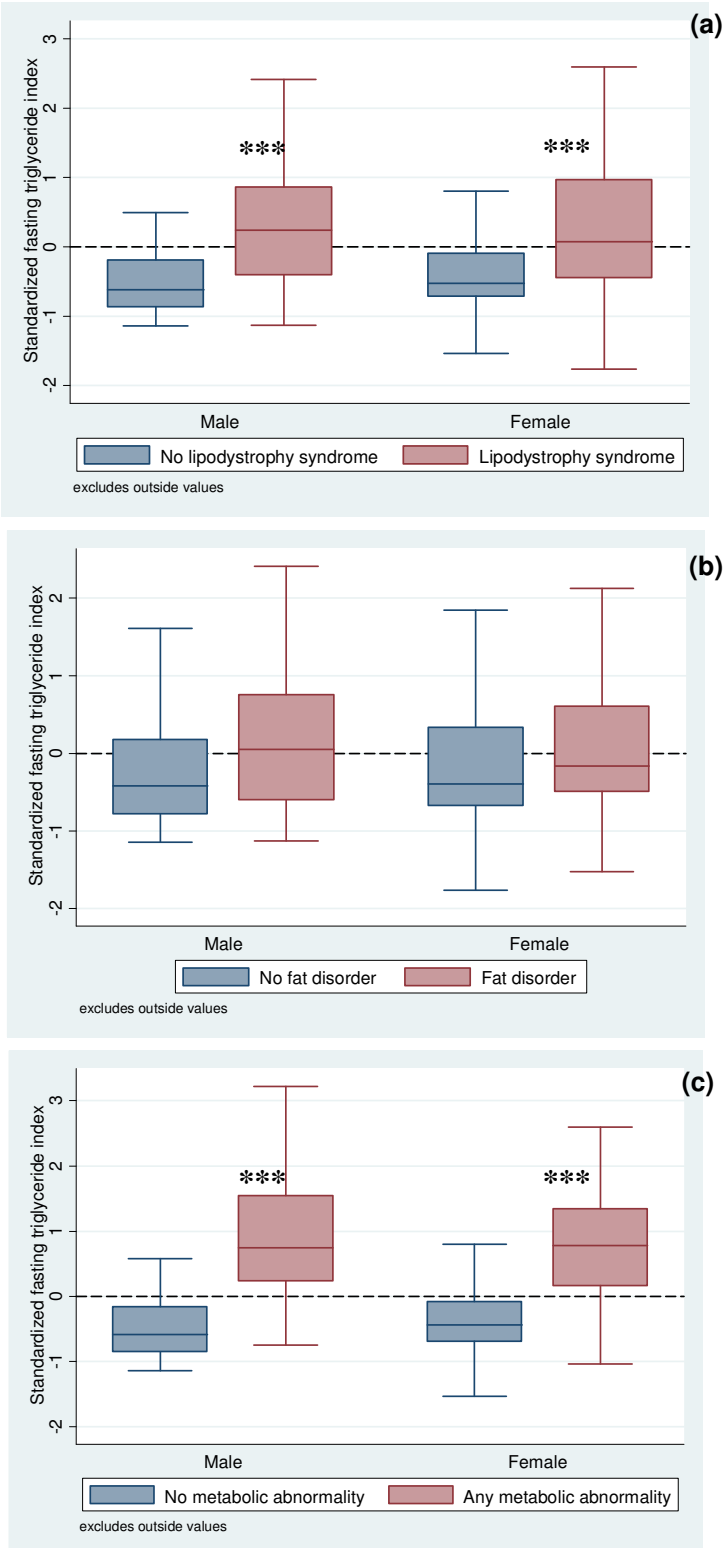
Thus it appears that older children may have increased levels of fasting triglyceride compared to younger children, with LS possibly having a greater effect on this relationship in males. However, while subjects with lipodystrophy had increased levels of fasting triglyceride compared to subjects without, differences in serum concentrations of fasting triglyceride were not significantly difference in subjects with body fat alterations compared to those without.

Figure 3-20: Total fasting triglyceride across age groups: (a) median fasting triglyceride, and (b) median standardized fasting triglyceride



Boxes demark 25th and 75th percentiles (2nd and 3rd quartile) and lines indicate adjacent values (most extreme values within 1.5 interquartile range of the nearest quartile) of the distribution within each age band. Values outside adjacent values excluded for clarity. Fasting triglyceride: *n* =311, and standardized fasting triglyceride: *n* = 309. No significant difference in standardized fasting triglyceride was seen between males and females in 2-3, 4-5, 6-7, 8-9, 10-11, and 12-13, year olds(*p* > 0.05): significant difference seen in 14-15 and 16-18 year olds (*p* < 0.05).

Figure 3-21: Comparison of median standardized fasting triglyceride, stratified by sex, between subjects with/without (a) lipodystrophy syndrome, (b) fat alteration, and (c) metabolic abnormality



Boxes demark 25th and 75th percentiles (1st and 3rd quartile) and lines indicate adjacent values (most extreme values within 1.5 interquartile range of the nearest quartile) of the distribution within each age band. Values outside adjacent values excluded for clarity. Comparison of median standardized fasting triglyceride ratio between subjects with outcome and without outcome: *$p < 0.01$, **$p < 0.01$, ***$p < 0.001$

3.10.4 Fasting insulin

Fasting insulin was available for almost half of the study population (199/426). Furthermore, additional subjects had missing data required for calculation of age and gender standardized fasting insulin: thus this was estimated in 46.0% of the population. Median fasting insulin levels were higher in males compared to females aged less than 7 years, with the converse being true for older subjects (Appendix C: Figure C-24). Furthermore, median levels increased with age.

Greater heterogeneity was seen in subjects with LS compared to those without (Appendix C: Figure C-26): however, this inference is clouded by the fact that fewer observations were available. Fractional polynomial modelling suggests that subjects who were older at recruitment tended to have higher levels of fasting insulin than subjects who were younger. Furthermore, these curves suggested that this increase was more pronounced in subjects with LS compared to those who did not. A jump in fasting insulin was seen at specific ages (subjects >10 years in females, and subjects>12 years in males), indicating that puberty may be an important time, in terms of serum insulin levels, in children with LS.

The fractional polynomial models for males and females raised age to different powers: all coefficients were significant in each the model for females, while all but one coefficient was significant in the model for males (Appendix C: Table C-19). This indicates that both sex and LS influence the relationship between age at recruitment and fasting insulin.

No significant differences were seen in standardized insulin measures between subjects with/without LS, metabolic abnormality, or body fat disorder (Appendix C: Figure C-27): in the majority of cases, subjects with an LS outcome had increased median values of standardized insulin compared to subjects without the outcome.

3.10.5 Fasting glucose

Data on fasting glucose was missing for 121 subjects, and a further 6 subjects were missing sex information. Thus fasting glucose was available for 71.6% of the study population while age and gender standardized fasting glucose could be calculated for 70.2%. Boxplots of both actual and standardized fasting glucose showed no significant differences by either age or gender (Appendix C: Figure C-29).

Heterogeneity between observations of age at recruitment and fasting glucose were similar amongst subjects with LS and without LS (Appendix C Figure C-31). Fractional polynomial models were similar in males and females without LS (Appendix C: Table C-21): regression functions oscillated with three peaks occurring over the age bracket, occurring slightly earlier in females compared to males. However, regression functions for subjects with LS oscillated less, with values of 75-80mg/dL of glucose seen across all age groups.

The structure of the fractional polynomial models was different between genders and also with LS status, with all coefficients being statistically significant except for those in the model for females with LS. This may be indicative of both sex and lipodystrophy having an influence on age at recruitment and levels of fasting glucose.

There were no significant differences in median standardized fasting glucose between subjects with LS compared to those without, in subjects with fat disorder compared to those without, and in those with metabolic abnormality compared to those without (Appendix C: Figure C-32).

3.11 Key points

- 426 children and adolescents (70% white ethnicity) were recruited into the cohort, with median age 12.2 years (IQR: 9.0, 15.0)

- 76% of participants showed no evidence of immunosuppression and 90% had no symptomatic disease at recruitment, while 32% had experienced either severe immunosuppression or clinical disease during their lifetime.

- Over half (56%) of subjects on ART at recruitment were being treated with PI-based HAART, and a third were being treated with NNRTI-based HAART.

- 29 subjects were ART-naïve (median age: 10.48 years, IQR: 7.77, 15.26)

- Prevalence of lipodystrophy syndrome at recruitment was 56.5% (95% CI: 51.7, 61.3), with half of cases defined by body fat alterations alone, a quarter by metabolic abnormality alone, and a quarter defined by both.

- Prevalence of body fat alterations at recruitment was 43.3% (95% CI: 37.0, 46.4): one third defined by lipohypertrophy alone, one third with lipoatrophy, and one third defined by both. Lipoatrophy occurred most commonly in the face and lipohypertrophy occurred most frequently in the trunk.

- Half (51%) of the 176 subjects with body fat alterations were affected in three or more locations.

- Trunk and neck lipohypertrophy occurred significantly more often in females compared with males, while age-specific differences in arm, leg and buttock lipoatrophy was seen in both males and females.

- Prevalence of metabolic abnormality at recruitment was 26.0% (95% CI: 21.8, 30.3): 44% of cases defined by fasting hypertriglyceridemia alone, 30% by hypercholesterolemia, 4% by glucose non-tolerance alone, and 23% by some combination of the three.

- There was evidence to suggest LS is more influential in serum concentrations of cholesterol in females but not males, whereas fasting triglyceride may be more effected by LS in males.

4. Fat alterations at recruitment

4.1 Objectives

The main objective of this chapter was to identify risk factors associated with prevalent body fat alterations at recruitment. Specific objectives were to:

- Estimate prevalence of (i) any fat alterations, (ii) lipohypertrophy, (iii) lipoatrophy, and (iv) both lipohypertrophy and lipoatrophy occurring together across socio-demographic, clinical and treatment groups

- Identify risk factors for each outcome fitting univariable logistic regression models

- Determine multivariable models of factors associated with each fat alterations outcome, including use of individual antiretroviral drugs, and to refit this in order to explore the effects of inclusion of ART class exposure, or of ART regimen (e.g. PI-based HAART, triple class) in place of individual drug exposure

4.2 Specific methods

Logistic regression modelling was used to identify potential risk factors for body fat alterations outcomes. The binary outcome measures are presented in Box 4-1. This was a cross-sectional analysis using data collected at recruitment (n = 428).

Box 4-1: Outcomes investigated in this chapter

Fat alterations outcomes
Any fat alterations
Any lipohypertrophy
Any lipoatrophy
Concurrent lipoatrophy and lipohypertrophy

Definitions of outcomes are given in Box 2-1in Chapter 2

4.2.1 Estimation of prevalence

Prevalences of each outcome by potential risk factors (e.g. ethnicity, specific ART drug, etc.) were estimated with respect to the numbers of subjects with complete data for that factor from the total study population. Associations between prevalence of outcomes and demographic and clinical characteristics were investigated using χ^2 tests.

4.2.2 Univariable regression modelling

The associations of potential risk factors and each outcome (Box 4-1) were investigated by fitting logistic regression models. The odds ratio (OR) measuring the association between each explanatory variable (Table 2-1 in Chapter 2) and outcome was estimated using univariable models.

4.2.3 Multivariable regression modelling

Three categories of multivariable models for each outcome were fitted yielding adjusted odds ratios (AOR): (i) models for the outcomes using ART classes as explanatory variables together with non-treatment related variables, (ii) as (i) but using specific ART drugs instead of class, and (iii) models using type of ART (specific cART and mono-therapy) as an explanatory variable. The first two sets of models were fitted using a backward stepwise approach (Section 2.8.6 in Chapter 2), while the last was intuitively adjusted for potential confounders. Table 2-1 in Chapter 2 illustrates the explanatory variables investigated in the stepwise process.

Multivariable models examining the role of type of cART (i.e. NRTI mono-therapy, PI-based HAART, NNRTI-based HAART, and triple class therapy) on fat alterations outcomes were not

determined using stepwise variable selection, but only included factors chosen *a priori*. These factors were age, total duration of ART, ethnicity and maximum CDC-clinical status, and the random effect for clinical site. The latter two variables were included in the models as they were statistically significant in univariable analysis and multivariable models (i.e. ethnicity was significant in multivariable models for all fat alterations outcomes except lipohypertrophy, and maximum CDC-defined clinical condition was significant in models for any body fat alterations and lipohypertrophy).

Three sets of sensitivity analyses were conducted. In the first, the final multivariable models were refitted but using moderate/severe outcomes (as opposed to the presence of the outcomes summarized in Box 4-1). In the second, the backward stepwise covariate selection was conducted but using ever-use of specific ART drugs as explanatory variables (as opposed to current-use at recruitment): these models used presence of the outcomes summarized in Box 4-1. The third set of sensitivity analyses repeated the backward stepwise selection process, but the threshold for statistical significance was set at 10%. Sensitivity analyses are included in Appendix D.

4.3 Results

4.3.1 Prevalence of body fat alterations outcomes by subject characteristics

There were significant differences in prevalence of body fat alterations outcomes by sex, age group, ethnicity, CDC-defined immune stage at recruitment, maximum CDC clinical stage, hepatitis-C co-infection, Tanner score for puberty, and with the specific ART drugs lamivudine, tenofovir, stavudine, zidovudine and efavirenz (Table 4-1).

Prevalence of lipohypertrophy was significantly higher among females (33%, n = 69) compared to males (22%, n = 43), but significant sex differences were not seen for other body fat alterations outcomes (Table 4-1). There was a significant ($p < 0.001$) association between increasing age and greater prevalence of fat alterations. Subjects of White ethnicity had the highest prevalence of all outcomes compared with other ethnic groups, with almost half of White children having body fat alterations (49%, n = 137): differences in prevalence between ethnic groups were significant for all outcomes ($p \leq 0.01$). While prevalence of body alterations was higher in subjects co-infected with hepatitis-C compared with uninfected subjects, this was only significant ($p \leq 0.012$) for any body fat alterations, and lipohypertrophy.

There were significant differences in the prevalence of any body fat alterations ($p < 0.001$), lipohypertrophy ($p = 0.010$), and lipoatrophy ($p = 0.004$) between subjects with differing Tanner score (Table 4-1): there was a clear trend of increased prevalence in subjects who had completed puberty compared to those undergoing puberty, and increased prevalence in those undergoing puberty compared to pre-pubescent subjects, with regard to both body fat alterations and lipohypertrophy. Indeed prevalence of any fat alterations was over 40% in subjects undergoing or having completed puberty.

There were significant differences in the prevalence of any body fat alterations ($p = 0.033$) and of lipohypertrophy ($p = 0.030$) with current immune status: prevalence was highest among subjects who showed no evidence of immunosuppression and lowest in those with moderate immunosuppression (Table 4-1). When considering nadir immune stage for both these outcomes, highest prevalence was seen in subjects with a history of severe immunosuppression, but this was not statistically significant ($p \geq 0.066$). Significant ($p \leq 0.007$) differences were seen in prevalence of any body fat alterations, lipohypertrophy, and lipoatrophy with maximum CDC clinical stage: prevalence in subjects who had been symptomatic at some point during their lifetime was at least 35%, and prevalence in subjects who had always been asymptomatic was at most 30% for each of these outcomes. No significant association was seen between current clinical condition and any body fat alteration outcome. Subjects with undetectable levels had a higher prevalence than those with detectable viral load though this difference did not reach statistical significance ($p \geq 0.341$).

Table 4-1: Prevalence of fat alterations at recruitment by participant characteristic

		Body fat alterations		Lipohypertrophy		Lipoatrophy		Combined lipohypertrophy and lipoatrophy	
		%	*n/N*	*%*	*n/N*	*%*	*n/N*	*%*	*n/N*
Demographic factors									
Sex	**Male**	39	77/199	22*	43/199	29	57/198	12	23/199
	Female	44	93/212	33*	69/211	26	55/211	15	31/212
Age (years)	**2-6**	16***	7/43	12*	5/43	7**	3/43	2*	1/43
	7-11	37***	37/100	26*	26/100	21***	21/98	10*	10/100
	12-18	50***	111/223	32*	70/222	35**	78/223	17*	37/223
Ethnicity	**Black**	22***	24/107	17*	18/107	9***	10/106	4***	4/107
	White	49***	137/281	32*	90/280	34***	95/280	17***	48/281
	Other	29***	5/17	24*	4/17	12***	2/17	6***	1/17
Country of residence	**Italy**	43***	118/272	28	75/271	26***	71/270	6***	5/84
	Belgium	26***	22/84	19	16/84	13***	11/84	10***	28/272
	Poland	55***	36/66	36	24/66	53***	35/66	35***	23/66
Infection factors									
CDC immune stage at recruitment	**Stage 1**	45*	145/321	29	94/320	31*	99/319	15	48/321
	Stage 2	30*	25/84	21	18/84	17*	14/84	8	7/84
	Stage 3	35*	6/17	18	3/17	24*	4/17	6	1/17
Nadir CDC immune stage	**Stage 1**	43	68/158	25	39/157	31	49/158	13	20/158
	Stage 2	38	67/178	30	53/178	22	39/177	14	25/178
	Stage 3	48	41/86	27	23/86	34	29/85	13	11/86
CDC clinical stage at recruitment	**N+A**	41	140/344	27	91/343	26	90/343	12	41/344
	B	48	10/21	29	6/21	38	8/21	19	4/21
	C	27	3/11	27	3/11	18	2/11	18	2/11

		Body fat alterations		Lipohypertrophy		Lipoatrophy		Combined lipohypertrophy and lipoatrophy	
		%	*n/N*	*%*	*n/N*	*%*	*n/N*	*%*	*n/N*
Maximum	**N+A**	30***	51/169	20**	34/169	19**	32/168	9	15/169
CDC clinical	**B**	50***	72/143	35**	49/142	34**	48/143	17	5/143
stage	**C**	52***	45/*87	34**	30/87	34**	29/86	16	14/87
Viral load	**≤50**	52	45/87	29	70/57	29	69/241	14	23/177
(copies/ml)	**>50**	39	69/177	25	44/176	27	48/177	13	23/177
Other factors									
Hepatitis C	**Uninfected**	40*	157/389	26*	102/388	27	106/387	13	51/389
co-infection	**Infected**	67*	16/24	50*	12/24	38	9/24	21	5/24
Tanner score	**I**	27***	36/135	19*	25/135	17**	23/134	9	121/135
for puberty	**II-IV**	47***	68/145	28*	41144	34**	50/145	16	23/145
	V	50***	47/94	36*	34/94	30**	28/94	16	15/94
Antiretroviral therapy									
ART naive	**No**	43*	170/394	28	110/393	29*	115/392	14	55/394
	Yes	21*	6/28	18	5/28	7*	2/28	4	1/28
NRTI									
Any NRTI	**Not current**	23*	7/30	20	6/30	10*	3/30	7	2/30
	Current	44*	159/360	28	100/359	30*	109/358	14	50/360
Didanosine	**Not current**	43	146/343	27	93/343	29	98/341	13	45/343
	Current	43	20/47	13	13/46	30	14/47	15	7/47
Lamivudine	**Not current**	45	65/144	31	45/144	33	47/144	19*	27/144
	Current	41	101/246	25	61/245	27	65/244	10*	25/246
Tenofovir	**Not current**	40	114/287	25	71/286	26	75/285	11*	32/287
	Current	50	52/103	34	35/103	36	37/103	19*	20/103
Stavudine	**Not current**	39***	136/364	26	89/345	26**	91/345	13	44/346
	Current	68***	30/44	39	17/44	49**	21/43	18	8/44
Zidovudine	**Not current**	46*	132289	30	86/288	32*	92/288	16*	46/289
	Current	34*	34/101	20	20/101	20	20/100	6*	6/101
NNRTI									
Any NNRTI	**Not current**	42	96/231	26	59/230	28	64/257	12	27/231
	Current	49	96/231	32	42/131	35	46/131	18	24/231

		Body fat alterations		Lipohypertrophy		Lipoatrophy		Combined lipohypertrophy and lipoatrophy	
		%	*n/N*	*%*	*n/N*	*%*	*n/N*	*%*	*n/N*
Efavirenz	**Not current**	39*	117/297	25*	73/296	26*	76/295	11**	32/297
	Current	53*	49/93	35*	33/93	39*	36/93	22**	20/93
PI									
Any PI	**Not current**	41	71/173	25	44/173	27	46/173	11	19/173
	Current	44	95/217	29	62/216	31	66/215	15	33/217
Type of drug therapy at recruitment	**PI-based HAART**	42	86/204	27	55/203	29	58/202	13**	27/204
	NRTI mono-therapy	38	11/29	17	5/29	24	7/29	3**	1/29
	NNRTI-based HAART	47	54/116	29	34/116	32	37/116	15**	17/116
	Triple class	69	9/13	54	7/13	62	8/13	46**	6/13

Association between participant characteristic and fat alterations (prevalence) investigated using χ^2 test: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$. CDC-defined immune status - stage 1: no immunosuppression, stage 2: moderate immunosuppression, stage 3: severe immunosuppression. CDC-defined clinical status - N + A: no symptoms/asymptomatic, B: moderate symptoms, C: severe

In all classes of ART, the prevalence of fat alterations was greater among subjects currently on the class at recruitment, compared to subjects not on the class (Table 4-1) Almost half of subjects currently on NRTIs had body fat alterations compared to almost one quarter of subjects not on these drugs (p = 0.027). Similarly, 30% of subjects on NRTI had lipoatrophy, in contrast to 10% of subjects not on these drugs (p = 0.018). While the size in the difference in prevalence was not as great, subjects who had ever been on NRTI had significantly higher prevalence of both any fat alterations, and lipoatrophy, compared to subjects who had never been on NRTI (Table 4-2). However current use of any ART was highly correlated with current use of NRTI: only two subjects who were on ART at recruitment were not on NRTI. Investigating association between NRTI and body fat alteration outcomes excluding ART naïve subjects showed no significant association (Table D-1in Appendix D).

Almost one in five of subjects on NNRTI at recruitment had combined lipoatrophy and lipohypertrophy compared to 12% not on this class (p = 0.039). However, none of the differences in fat alteration prevalence observed with recruitment PI use were statistically significant. Nevertheless, subjects who were on either NNRTI or PI at recruitment had higher prevalence of all fat alterations outcomes compared to subjects who had never been on these drugs (Figure 4-1). Furthermore, subjects who had ever been on NNRTI or PI had significantly increased prevalence of all body fat abnormality outcomes compared to subjects who had never been on these antiretroviral classes (Table 4-2).

Significant differences were seen in the prevalence of any body fat alterations, and lipoatrophy between subjects who were ART-naïve and those who had been treated (Table 4-2): this suggests that the significance differences seen in prevalence between these outcomes by each category of ART may be driven by the numbers of subjects who were treatment naïve. Indeed, exclusion of ART naïve subjects from these analyses resulted in non-significant associations between all classes of ART used at recruitment and body fat alteration outcomes (Table D-1 in Appendix D).

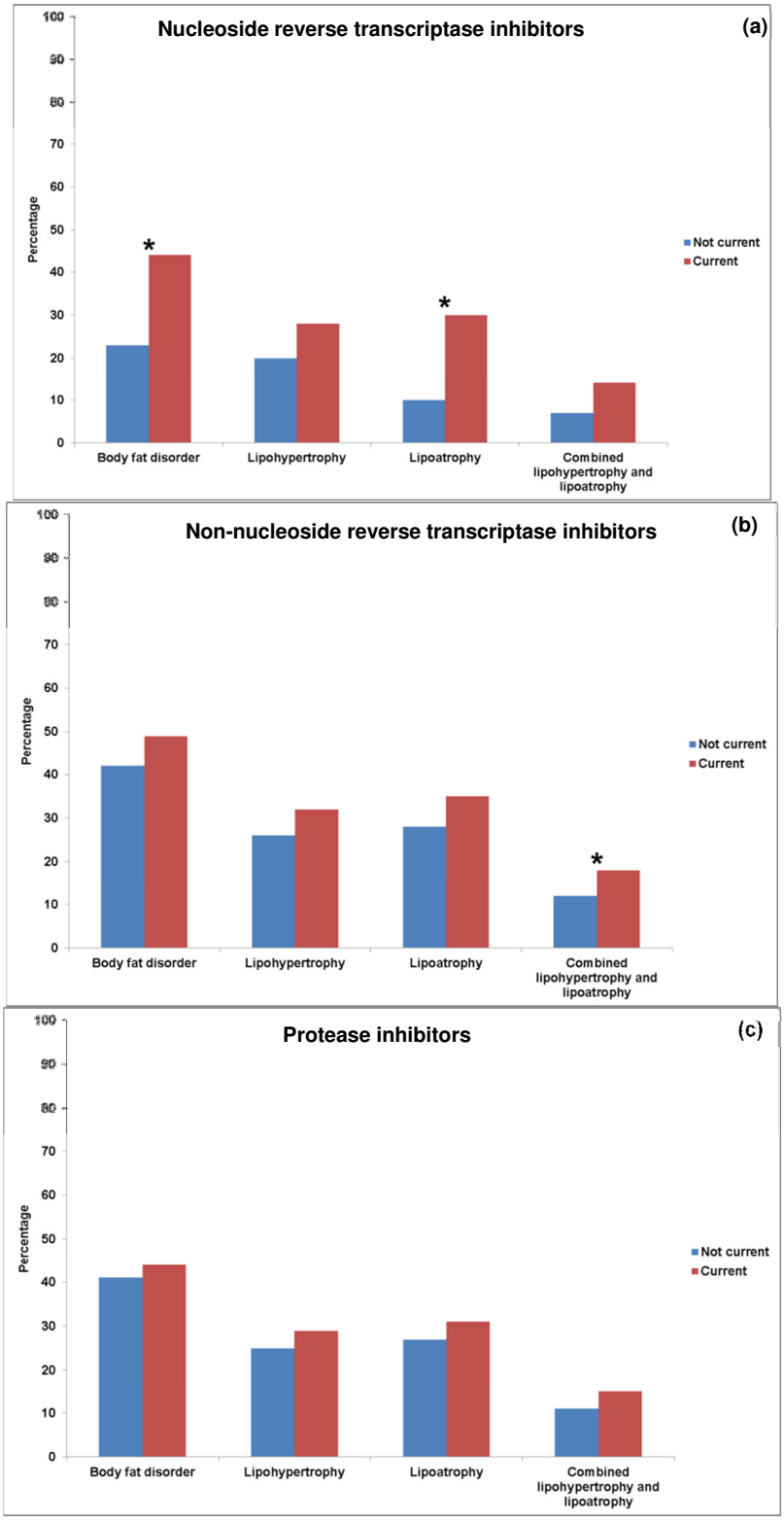
Table 4-2: Prevalence of fat alterations at recruitment by ever use of categories of antiretroviral therapy

		Body fat alterations		Lipohypertrophy		Lipoatrophy		Combined lipohypertrophy and lipoatrophy	
		%	*n/N*	*%*	*n/N*	*%*	*n/N*	*%*	*n/N*
NRTI	**Never use**	21*	6/28	18	5/28	7*	2/28	4	1/28
	Ever use	43*	170/394	28	110/393	29*	115/392	14	55/394
NNRTI	**Never use**	34**	60/179	22*	40/179	30**	36/177	9*	16/179
	Ever use	48**	116/243	31*	75/242	33**	81/243	16*	40/243
PI	**Never use**	27**	27/95	17**	16/95	17**	16/95	5**	5/95
	Ever use	46**	149/327	30**	99/326	31**	101/325	16**	51/327
ART naïve	**Treated**	42	170/394	28	110/393	29*	115/392	14	55/394
	Naïve	21*	6/28	18	5/28	7*	2/28	4	1/28

Association investigated using χ^2 test: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

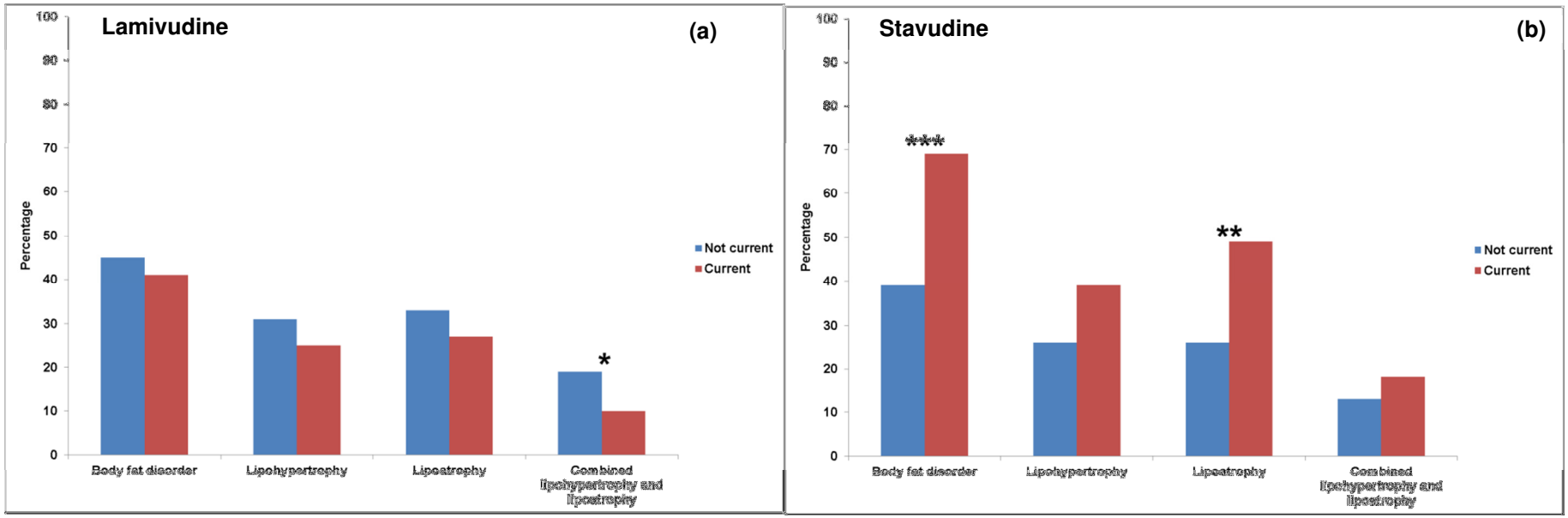
Significant differences in prevalence of body fat alterations between subjects on a specific ART drug and those not on that drug were only seen for five drugs: lamivudine (concurrent lipoatrophy and lipohypertrophy: $p = 0.016$), stavudine, (body fat alterations, lipoatrophy: $p \leq 0.002$) zidovudine (body fat alterations, lipohypertrophy, concurrent lipoatrophy and lipohypertrophy: $p \leq 0.051$), tenofovir, (concurrent lipoatrophy and lipohypertrophy: $p = 0.034$) and efavirenz (all outcomes: $p \leq 0.041$), as illustrated in Figure 4-2a, Figure 4-2b, and Figure 4-3b. No other significant associations were found.

Figure 4-1: Association between class of antiretroviral therapy and body fat alterations outcomes



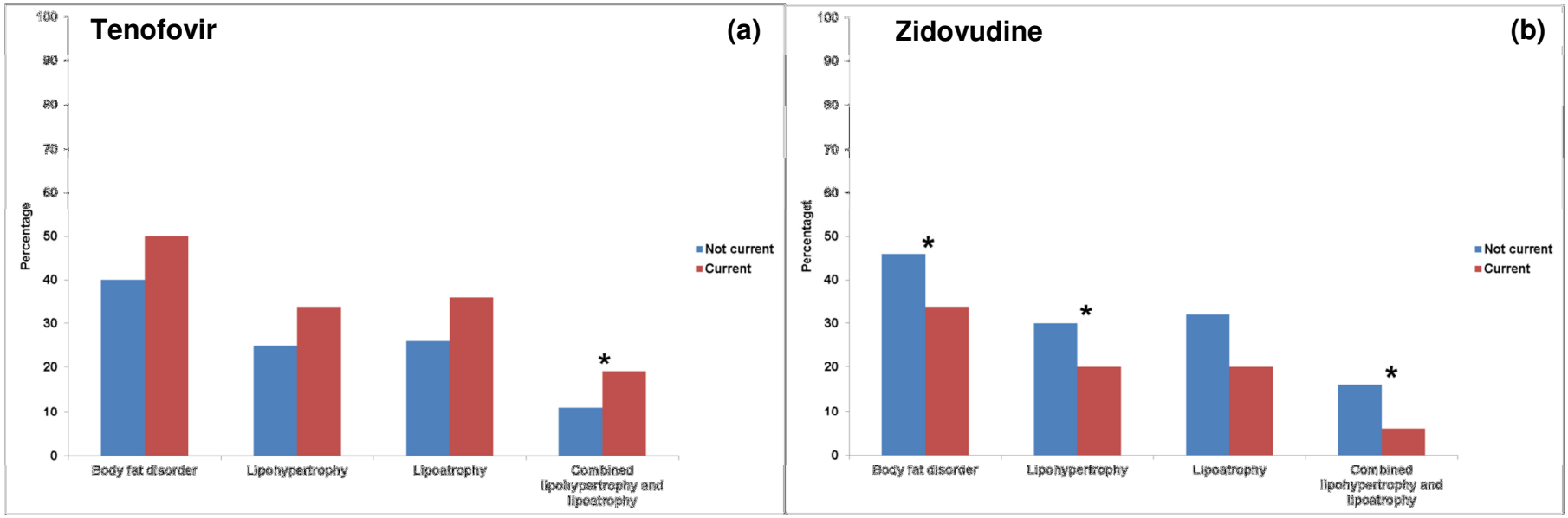
Association between body fat alterations and (a) nucleotide reverse transcriptase inhibitors, (b) non-nucleotide reverse transcriptase inhibitors, and (c) protease inhibitors. Not current includes ART-naïve subjects. Association investigated using χ^2 test: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

Figure 4-2: Prevalence if fat alterations by current use of (a) lamivudine, and (b) stavudine



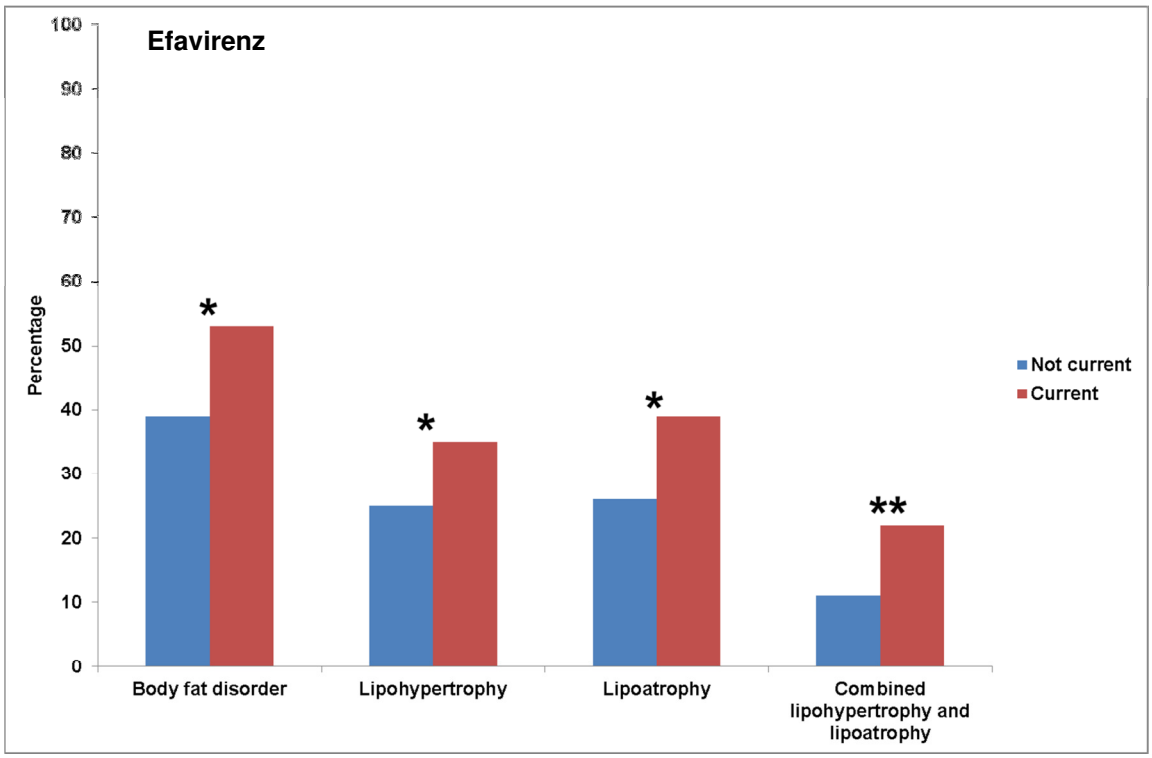
Association investigated using χ^2 test: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

Figure 4-3: Prevalence of fat alterations by current use of (a) tenofovir, and (b) zidovudine



Association investigated using χ^2 test: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

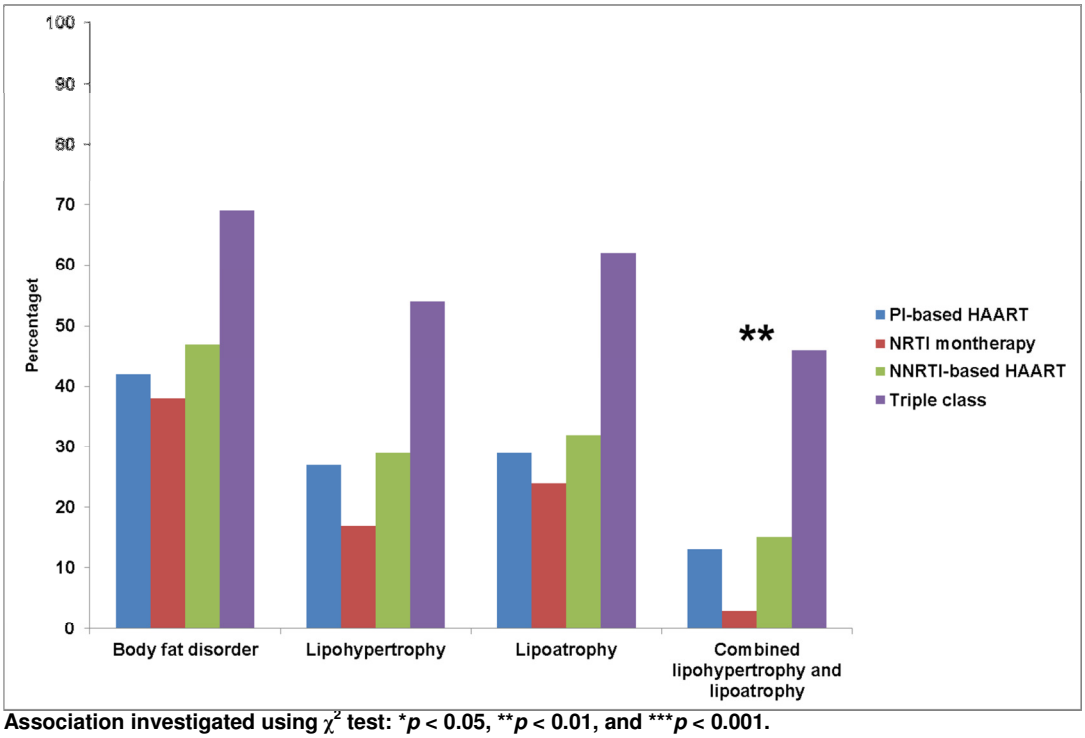
Figure 4-4: Prevalence of fat alterations by current use of efavirenz



Association investigated using χ^2 test: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

When considering type of ART regimen, across all fat alteration outcomes the highest prevalence was seen in subjects on triple class therapy at recruitment (n = 14), with prevalences being similar in subjects on PI-based HAART (n = 204) and NNRTI-based HAART (n = 117), as illustrated in Figure 4-5. Subjects on NRTI mono-therapy (n =29) had the lowest prevalence of fat alterations. However, only the association between recruitment ART regimen and concurrent lipoatrophy and lipohypertrophy was statistically significant (p = 0.003).

Figure 4-5 : Prevalence of fat alterations outcomes at recruitment by antiretroviral therapy regimen



Association investigated using χ^2 test: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

4.3.2 Univariable analyses of factors associated with body fat alterations

A significant increased risk of any body fat alterations was seen with increasing age: compared to 2-6 year olds, 7-11 year olds had an almost 3-fold increase in risk, and 12-18 year olds had an almost 5-fold increase in risk (Table D-2 in Appendix D). However, the risks associated with undergoing puberty (OR: 2.43, 95% CI: 1.47, 4.01), and completed puberty (OR: 2.75, 95% CI: 1.58, 4.79), compared to pre-pubescence, were equivalent with each other. White ethnicity was associated with a 3-4 fold increased risk compared to Black ethnicity ($p < 0.001$), while a history of CDC-defined clinical disease was associated with a 2-3 fold increased risk ($p \leq 0.001$), and co-infection with hepatitis-C was associated with a 2-3 fold increased risk ($p = 0.015$). Use of any NRTI at recruitment (OR: 2.60, 95% CI: 1.09, 6.21), and specifically stavudine (OR: 3.31, 95% CI: 1.69, 6.47) were significant risk factors for body fat alterations. Both NRTI and stavudine were significant risk factors for body fat alterations in univariable analysis despite only 30% (n/N = 30/159) of the total number of subjects currently on NRTIs being on stavudine. Additionally, current efavirenz was associated with a 70% increase in risk of any body fat alterations ($p = 0.024$).

Factors associated with a significant decrease in risk of body fat alterations included moderate immunosuppression at recruitment, (OR: 0.51, 95% CI: 0.31, 0.86), and current zidovudine at recruitment (OR: 0.60, 95% CI: 0.38, 0.97). There was no statistically significant association found between fat alterations and sex, nadir CDC-defined immune stage, recruitment CDC clinical stage, detectable viral load or current PI use.

With respect to lipohypertrophy, females had a significant 76% increased risk compared with males (Table D-2 in Appendix D). Furthermore, the 12-18 year old group had an increased risk that was over 3-times that for 2-6 year old group, and White ethnicity was associated with a 2-fold increased risk compared to Black ethnicity ($p < 0.05$). Undergoing puberty (Tanner II-IV) and completion of puberty (Tanner V) were associated with an increased risk of lipohypertrophy, compared to a pre-pubescent state (Tanner I): OR: 1.75, 95% CI: 1.00, 3.08, and OR: 2.49, 95% CI: 1.36, 4.56 respectively. Symptomatic maximum clinical stage was associated with a significant 2-fold increase in risk, while hepatitis C co-infection was associated with a 3-fold increase in risk ($p \leq 0.015$): the size of these associations was similar to those seen with any fat alterations. The only specific ART drug to be significantly associated with lipohypertrophy was efavirenz, which was associated with a 68% increase in risk. In comparison to PI-based HAART, triple class therapy was associated with a significant increased risk of lipohypertrophy (OR: 3.14, 95% CI: 1.01, 9.75). Immune stage, clinical stage at recruitment, detectable viral load, and current use of NRTI or PI did not show a statistically significant association with lipohypertrophy.

In common with any fat alterations and lipohypertrophy, increasing age group was associated with an increase in risk of lipoatrophy: in comparison to the 2-6 year old group, the 7-11 year old group had a 3-4 fold risk, and the 12-18 year old group had a 6-7 fold risk ($p \leq 0.039$), as illustrated in Table 4-3. Similarly, black ethnicity (OR: 4.93, 95% CI: 2.46, 9.89), and both undergoing (OR: 2.54, 95% CI: 1.44, 4.47) or completion (OR: 2.05, 95% CI: 1.09, 3.84) of puberty were also significant risk factors for lipoatrophy (as with other fat alterations outcomes).

HIV-related factors associated with a significant risk of lipoatrophy were immune stage at recruitment and maximum clinical stage: symptomatic maximum clinical status was associated with a 2-3 fold increased risk; while moderate immunosuppression at recruitment was associated with a 56% reduced risk. Indeed, moderate immunosuppression was associated with a decrease in risk of all body fat alterations outcomes, but was only statistically significant with respect to lipoatrophy.

Use of any NRTI at recruitment was associated at a significant increased risk of lipoatrophy (OR: 3.94, 95% CI: 1.17, 13.26), as was stavudine (OR: 2.66, 95% CI: 1.40, 5.07). However zidovudine was associated with a 47% decreased risk of lipoatrophy ($p = 0.025$). While current NNRTI was associated with a non-significant increase in risk, current efavirenz was associated with an 82% increased risk ($p = 0.017$). Moreover, triple class therapy at recruitment was associated with a significant, almost 4-fold increased risk of lipoatrophy compared to PI-based HAART.

As with other fat alterations outcomes, increasing age and ethnicity were associated with a significant increased risk of the combined lipohypertrophy and lipoatrophy occurring together. However, the size of the increased risk was greater than with other outcomes: 12-18 years of age was associated with an 8-9 fold risk compared to 2-6 years, and Black ethnicity was

associated with a 5-6 fold risk compared to white ethnicity (Table D-2 in Appendix D). Children who had ever experienced CDC stage B (moderate) symptoms had a two-fold increased risk of the combined sub-type (p = 0.026). While current NRTI use was associated with a non-significant increased risk of concurrent lipoatrophy and lipohypertrophy, current tenofovir was statistically significant (OR: 1.92, 95% CI: 1.04, 3.54). However, a significant reduced risk was associated with both current lamivudine (51%) and current zidovudine (33%) use. Use of any NNRTI, or efavirenz, at recruitment were both associated with a 2-fold increased risk of he combined phenotype ($p \leq 0.041$). However, the association seen with any NNRTI may be driven by efavirenz since 70.7% (n/N = 94/133) of the subjects on NNRTI's at recruitment were on this specific drug. The small number of children receiving triple class therapy had a more than 5-fold statistically significant increased risk compared with those on PI-based HAART.

Viral load, and both CDC-defined immune and clinical status at recruitment, showed consistent associations across body fat alterations outcomes: both immunosuppression and detectable viral load were associated with a decrease in risk, and moderate clinical symptoms were associated with an increase in risk. Interestingly, despite all three covariates being (direct and indirect) markers of HIV infection, where one covariate was statistically significant with respect to an outcome, the remaining two were non-significant. Both detectable viral load and immunosuppression can occur as a result of treatment failure or the absence of treatment, and thus the protective effect against fat alterations seen in univariable analyses may be confounded by the increased risk associated with ART.

Ever use of any ART was a significant risk factor for any body fat alterations (OR: 2.78, 95% CI: 1.10, 7.01) and lipoatrophy (OR: 4.38, 95% CI: 1.26, 23.11), as illustrated in Table D-3 in Appendix D. Ever use of didanosine, stavudine, efavirenz, ritonavir booster, and indinavir were all associated with a significant increased risk of all fat alterations outcomes. Ever use of tenofovir was associated with a significant ($p < 0.05$) increased risk of all outcomes except lipohypertrophy. While nelfinavir was associated with a significant increased risk of any body fat alterations, and saquinavir was associated with a significant increased risk of lipohypertrophy.

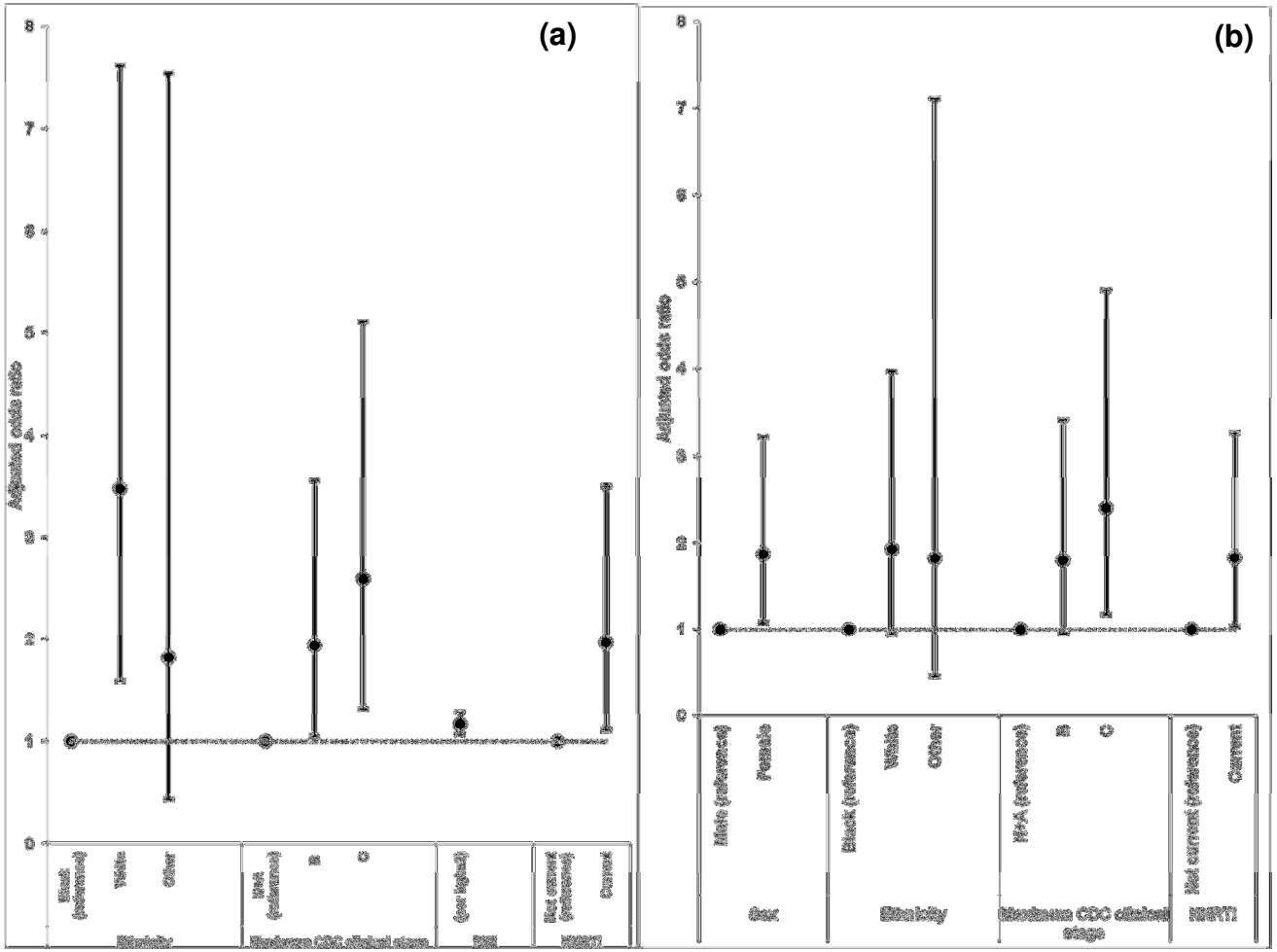
4.3.3 Multivariable models including antiretroviral therapy class

Use of NNRTI at recruitment was a positive risk factor in final multivariable models using classes of ART as explanatory variables (Figure 4-6 and Figure 4-7): this was consistent with univariable analyses where efavirenz was associated with increased risk of all outcomes (Table D-3 in Appendix D). An independent increased risk was seen for any fat alterations (AOR: 1.97, 95% CI: 1.11, 3.50, $p = 0.021$), lipohypertrophy (AOR: 1.83, 95% CI: 1.03, 3.26, $p = 0.039$) and lipoatrophy (AOR: 1.85, 95% CI: 1.01, 3.42, $p = 0.048$), while a 7-8 fold risk was seen for combined lipohypertrophy and lipoatrophy (AOR: 7.82, 95% CI: 1.97, 31.04, $p = 0.003$): these associations were all of greater magnitude than the ORs seen with efavirenz. Maximum CDC clinical stage was associated with increased risk in all models, but was only significant in the models for any fat alterations where stage B was associated with 2 fold risk (AOR: 1.94, 95% CI: 1.05, 3.56, $p = 0.034$), and for lipohypertrophy where stage C was associated with a 2-3 fold risk (AOR: 2.40, 95% CI: 1.17, 4.91, $p = 0.016$), versus an asymptomatic CDC stage: both increases in magnitude compared to the univariable analyses.

White ethnicity was also a consistent risk factor: this was significant for any body fat alterations (AOR: 3.48, 95% CI: 1.59, 7.62, $p = 0.002$), lipoatrophy (AOR: 5.08, 95% CI: 1.95, 13.27, $p = 0.001$) and combined lipohypertrophy and lipoatrophy (AOR: 4.28, 95% CI: 1.09, 16.79, $p = 0.037$), but non-significant for lipohypertrophy (AOR: 1.93, 95% CI: 0.94, 3.97, $p = 0.074$). As seen with efavirenz and NNRTI in univariable and multivariable analyses, the adjusted risk associated with White ethnicity was of greater magnitude compared to the unadjusted risk.

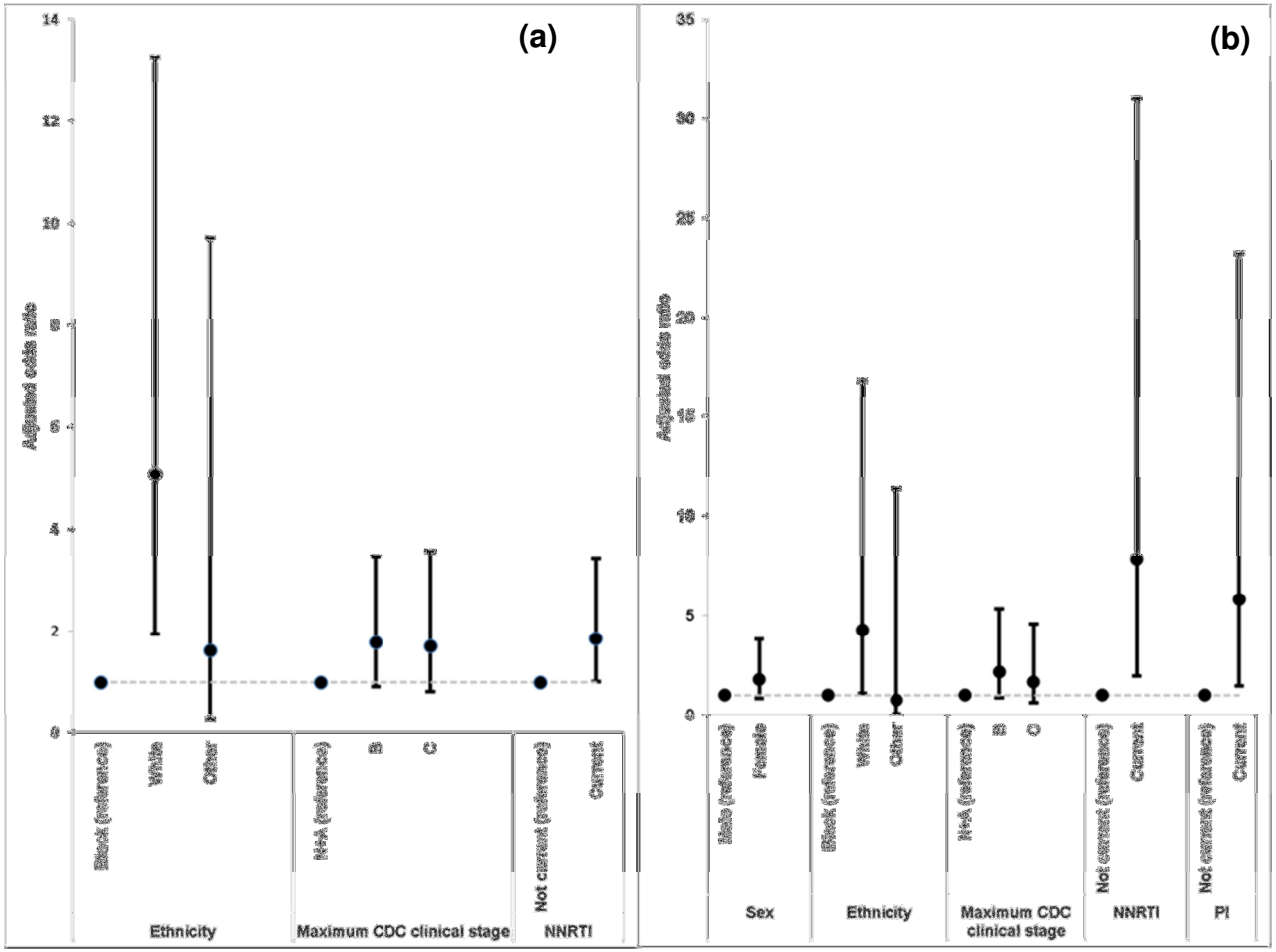
Females were at increased risk of lipohypertrophy (AOR: 1.87, 95% CI: 1.08, 3.22, $p = 0.025$, p) compared to males. A 2-fold increased risk of the combined phenotype was also associated with female sex, but this was non-significant. BMI was only a significant risk factor for any fat alterations, with a 17% increased risk for each m/kg^2 (AOR: 1.17, 95% CI: 1.07, 1.29, $p = 0.001$). Current PI was associated with the combined lipoatrophy and lipohypertrophy (AOR: 5.81, 95% CI: 1.46, 23.20 $p = 0.013$) despite not being significant in univariable analyses.

Figure 4-6: Final multivariable models for (a) any body fat alterations, and (b) lipohypertrophy using classes of antiretroviral therapy as explanatory variables



All models adjusted for age and maximum duration of ART use. CDC-defined clinical status - N + A: no symptoms/asymptomatic, B: moderate symptoms, C: severe symptoms

Figure 4-7: Final multivariable models for (a) lipoatrophy, and (b) combined lipohypertrophy and lipoatrophy, using classes of antiretroviral therapy as explanatory variables



All models adjusted for age and maximum duration of ART use. CDC-defined clinical status - N + A: no symptoms/asymptomatic, B: moderate symptoms, C: severe symptoms

4.3.4 Multivariable models including specific antiretroviral drugs
Body fat alterations

In the final multivariable model for any fat alterations (Table 4-3), stavudine at recruitment was associated with a significant 5-fold increased risk, and efavirenz was associated with a significant 2-fold increased risk. However, didanosine was associated with a non-significant reduction in risk (AOR: 0.42, 95% CI: 0.17, 1.01). Other factors associated with a significant risk were White ethnicity compared to Black ethnicity (AOR: 3.32, 95% CI: 1.49, 7.41), maximum CDC-clinical stage C compared to stage N/A (AOR: 2.34, 95% CI: 1.18, 4.65), and BMI (AOR: 1.17, 95% CI: 1.06, 1.28).

Similar significant associations were found between ethnicity, maximum CDC clinical status, BMI, and efavirenz (NNRTI) as in the ART class model for body fat alterations. Additional associations were made with stavudine (positive) and didanosine (negative) in the individual-drug multivariable model.

Didanosine did not show a significant association with any body fat alterations in either univariable or multivariable modelling. However, didanosine was retained in the final multivariable model because of its effect on the statistical significance of stavudine. Despite the reported toxicity of this ART combination[296,388], toxicities have been shown to be relatively rare in children[389]. Missing data for both didanosine and stavudine, and also interactions with other variables was investigated in the multivariable models: none of these factors were found to be statistically significant.

In sensitivity analysis, rerunning the final model with moderate/severe fat alterations as the outcome, only White ethnicity (AOR: 3.17, 95% CI: 1.09, 9.28) and BMI (AOR: 1.21, 95% CI: 1.09, 1.35) remained statistically significant as illustrated in the Table D-5 in Appendix D: no drug had a significant association to moderate/severe body fat alterations.

The final multivariable model incorporating ever-use of specific ART drugs (Table D-4 in Appendix D) contained White ethnicity (AOR: 2.56, 95% CI: 1.25, 5.23), BMI (AOR: 1.2, 95% CI: 1.09, 1.31), stavudine (AOR: 3.56, 95% CI: 2.08, 6.09), and efavirenz (AOR: 1.86, 95% CI: 1.10, 3.16), No factor was associated with a significant decrease in risk of body fat alterations

Table 4-3: Final multivariable model for fat alterations using specific antiretroviral drugs

		n	AOR	95% CI	*p*-value
Age	**Per year**	302	1.08	(0.99, 1.18)	0.102
Maximum duration of ART	**Per year**	302	1.03	(0.96, 1.12)	0.389
Ethnicity	**Black**	73	1		
	White	215	3.32	(1.49, 7.41)	0.003
	Other	14	1.95	(0.46, 8.27)	0.364
Maximum	**N/A**	121	1		
CDC clinical	**B**	110	1.87	(0.99, 3.52)	0.052
stage	**C**	71	2.34	(1.18, 4.65)	0.015
BMI	**Per kg/m^2**	302	1.17	(1.06, 1.28)	0.002
Stavudine	**Not current**	266	1		
	Current	36	5.40	(2.15, 13.56)	<0.001
Didanosine	**Not current**	261	1		
	Current	41	0.42	(0.17, 1.01)	0.052
Efavirenz	**Not current**	226	1		
	Current	76	1.93	(1.03, 3.62)	0.042

Model intuitively adjusted for age and duration of ART at recruitment: Adjusted odds ratios (AOR) and 95% confidence interval for fat redistribution by participant characteristic. CDC-defined clinical status - N + A: no symptoms/asymptomatic, B: moderate symptoms, C: severe symptoms

Lipohypertrophy

No specific ART drug remained statistically significant in the multivariable model for lipohypertrophy. In the adjusted model excluding any specific drug, both maximum CDC-defined clinical condition and BMI were associated with significantly increased risk (Table 4-4).

Furthermore, in contrast to the multivariable models using class of ART, ethnicity was not statistically significant (and so not included in the model), while BMI at recruitment was significant. (AOR: 1.46, 95% CI: 1.30, 1.63) Risk associated with maximum clinical symptoms was similar to that seen in univariable analysis.

Table 4-4: Final multivariable model for lipohypertrophy using specific antiretroviral drugs

		N	AOR	95% CI	*p*-value
Age	**Per year**	337	0.93	(0.85, 1.02)	0.127
Maximum duration of ART	**Per year**	337	0.99	(0.91, 1.07)	0.744
Maximum CDC clinical condition	**N/A**	139	1		
	B	121	2.08	(1.07, 4.05)	0.031
	C	77	2.24	(1.07, 4.69)	0.040
BMI	**Kg/m^2**	337	1.46	(1.30, 1.63)	<0.001

Model intuitively adjusted for age and duration of ART at recruitment: Adjusted odds ratio (AOR) and 95% confidence interval for lipohypertrophy by participant characteristic. CDC-defined clinical status - N + A: no symptoms/asymptomatic, B: moderate symptoms, C: severe symptoms

Only BMI (AOR: 1.51, 95% CI: 1.21, 1.75) remained statistically significant in the sensitivity analysis rerunning the final model with moderate/severe lipohypertrophy (Table D-5 in Appendix D). While history of severe clinical disease was associated with a 22% increase in risk, this was non-significant (p = 0.707). In contrast to the final model using current drugs at recruitment, a single specific ART drug was significant in the final model for lipohypertrophy using ever drug use: stavudine was associated with a 2-3 fold increase in risk (AOR: 2.53, 95% CI: 1.38, 4.63). The only other factor that was significant in this model was BMI (AOR: 1.46, 95% CI: 1.30, 1.64) as illustrated in the Table D-4 in Appendix D.

Lipoatrophy

Use of stavudine at recruitment was associated with an independent and significant 2-3 fold increase in risk of lipoatrophy in the final multivariable model (Table 4-5). Consistent with the previous models for any fat alterations and those using class of ART as explanatory variables, White ethnicity was associated with a significantly increased risk of lipoatrophy compared to Black ethnicity. (AOR: 4.01, 95% CI: 1.55, 10.38).

While children who had ever had moderate symptoms of HIV disease had a nearly 2-fold increased risk (p = 0.053), having a history of moderate immunosuppression was associated with a significant 58% reduction in risk of lipoatrophy. However, in previous univariable analysis, maximum clinical stage had been associated with a significant two-fold increased risk, while the risk associated with nadir CDC-define immune stage was non-significant. Nadir clinical condition appeared to be confounded by both age and maximum clinical condition acting in the opposite direction (negative confounding) in univariable analysis. In contrast to previous multivariable models using class of ART, age was a significant risk factor, with a 20% increase in risk of lipoatrophy for every year of age.

Table 4-5: Final multivariable model for lipoatrophy using specific antiretroviral therapy drugs

		n	AOR	95% CI	*p*-value
Age	**Per year**	303	1.20	(1.09, 1.32)	<0.001
Maximum duration of ART	**Per year**	303	1.03	(0.95, 1.11)	0.502
Ethnicity	**Black**	72	1		
	White	217	4.01	(1.55, 10.38)	0.004
	Other	14	1.35	(0.22, 8.34)	0.746
Maximum CDC clinical condition	**N/A**	120	1		
	B	111	1.95	(0.99, 3.85)	0.053
	C	72	1.58	(0.73, 3.45)	0.248
Nadir CDC immune-suppression	**Stage 1**	111	1		
	Stage 2	122	0.42	(0.21, 0.83)	0.012
	Stage 3	70	0.72	(0.33, 1.58)	0.410
Stavudine	**Not current**	268	1		
	Current	35	2.69	(1.12, 6.47)	0.027

Model intuitively adjusted for age and duration of ART at recruitment: Adjusted odds ratio (AOR) and 95% confidence interval for lipoatrophy by participant characteristic. CDC-defined clinical status - N + A: no symptoms/asymptomatic, B: moderate symptoms, C: severe symptoms. CDC-defined immune status - stage 1: no immunosuppression, stage 2: moderate immunosuppression, stage 3: severe immunosuppression.

The independent significant negative association with nadir immune status may occur as a result of confounding by association. Previous poor viral control may result in immunosuppression which may lead to ART treatment: in such a situation both poor nadir immune status and current ART is associated with lipoatrophy. Since there is no measure of viral load at the nadir clinical status, this confounding is difficult to investigate.

In sensitivity analysis modelling moderate/severe lipoatrophy, stavudine was associated with a non-significant increase in risk (AOR: 2.18, 95% CI: 0.77, 6.15), as illustrated in Table D-5 in Appendix D. However, both a history of moderate immunosuppression and age were significant risk factors, the former being associated with a reduced risk (AOR: 0.32, 95% CI: 0.14, 0.76), and the latter with an increased risk (AOR: 1.24, 95% CI: 1.09, 1.42), reinforcing the results summarized in Table 4-5.

The final multivariable model incorporating ever-use of ART contained both stavudine (AOR: 4.98, 95% CI: 2.58, 9.61), and efavirenz (AOR: 1.87, 95% CI: 1.05, 3.35). Furthermore, the association with White ethnicity in the ever-ART model was similar to that seen in the current-ART model, i.e. AOR: 3.87, 95% CI: 1.50, 10.01). Age was also associated with a significant increase in risk (AOR: 1.17, 95% CI: 1.06, 1.28). No other factor was statistically significant in the final model (Table D-4 in Appendix D).

Lipohypertrophy and lipoatrophy occurring together

Two drugs were significantly associated with a reduced risk of this concurrent lipoatrophy and lipohypertrophy: zidovudine and lamivudine, with 67% and 54% reductions in risk respectively. Both White ethnicity (4.15, 95% CI: 0.99, 17.45) and BMI (AOR: 1.20, 95% CI: 0.07, 11.70) were associated with an increase in risk, but this was only statistically significant in the latter (Table 4-6). All factors, with the exception of BMI, had a magnitude and direction similar to those seen in unadjusted analysis (Table D-3 in Appendix D).

Table 4-6: Final multivariable model for both lipohypertrophy and lipoatrophy occurring together, using specific antiretroviral therapy drugs

		n	AOR	95% CI	*p*-value
Age	**Per year**	317	1.04	(0.91, 1.19)	0.544
Maximum duration of ART	**Per year**	317	1.20	(1.06, 1.36)	0.003
Ethnicity	**Black**	82	1		
	White	220	4.15	(0.99, 17.45)	0.052
	Other	15	0.90	(0.07, 11.70)	0.939
BMI	**Per kg/m^2**	317	1.20	(1.06, 1.36)	0.003
Zidovudine	**Not current**	222	1		
	Current use	95	0.33	(0.12, 0.91)	0.032
Lamivudine	**Not current**	98	1		
	Current use	219	0.46	(0.21, 0.99)	0.047

Model intuitively adjusted for age and duration of ART at recruitment: Adjusted odds ratio (AOR) and 95% confidence interval (95% CI) for lipohypertrophy and lipoatrophy occurring together by participant characteristic.

White ethnicity remained a significant factor associated with an increased risk of moderate/severe combined lipohypertrophy and lipoatrophy in sensitivity analysis (AOR: 20.19, 95% CI: 1.40, 291.36). Furthermore, lamivudine was associated with a significant 94% reduction in risk (AOR: 0.06, 95% CI: 0.01, 0.58), as illustrated in (Table D-5 in Appendix D).

Further sensitivity analyses where the threshold to include covariates was increased to 10%. Categories of ART thus significantly associated with body fat alteration outcomes were similar to those seen in the models where inclusion significance was set at 5% (Appendix D: Table D-8). However, in the models investigating specific drugs: the models for any body alterations and lipoatrophy resulting from using 5% and 10% thresholds differed: at the 10% level, zidovudine was significantly associated with increased risk of body fat alterations, while efavirenz was significantly associated with increased risk of lipoatrophy (Appendix D: Table D-8).

In contrast to the model using current-ART, no factor in the final multivariable model for the combined phenotype using ever-ART was associated with a decrease in risk: age (AOR: 1.13, 95% CI: 1.00, 1.27), efavirenz (AOR: 2.38, 95% CI: 1.11, 5.12), didanosine (AOR: 3.44, 95% CI: 1.47, 8.07), ritonavir booster (AOR: 2.92, 95% CI: 1.16, 7.33), and indinavir (AOR: 4.00, 95%

CI: 1.04, 15.41) were all significant factors in the final model, as illustrated in Table D-4 in Appendix D.

The stepwise multivariable modelling investigating current ART at recruitment indicates that while NNRTIs were consistently associated with all body fat alteration outcomes, only one specific drug, efavirenz was significant for a specific outcome (any body fat alterations). Use of any PI was associated with increased risk of concurrent lipoatrophy and lipohypertrophy at recruitment, but no specific PI was associated with any outcome. While NRTI's as a whole were not associated with any outcome at recruitment, stavudine was associated with increased risk of any fat alterations, and lipoatrophy, didanosine with reduced risk of any alterations, and both zidovudine and lamivudine with reduced risk of the concurrent manifestation in multivariable models.

White ethnicity was a consistent independent risk factor for all body fat alteration outcomes excluding lipohypertrophy, and a history of clinical disease was associated with increased risk of both any fat alterations, and lipohypertrophy. In contrast to the models using current-ART at recruitment as explanatory variables, all statistically significant drugs in the ever-ART multivariable models were associated with an increased risk: ever-stavudine was associated with a 2-5 fold increased risk of any fat alterations, lipohypertrophy, and lipoatrophy, ever-efavirenz was associated with a <3-fold increased risk of any fat alterations, lipoatrophy, and the combined manifestation, while didanosine, ritonavir booster and indinavir were associated with an increased risk of the combined manifestation. Furthermore age was significantly associated with an increased risk of both lipoatrophy and lipohypertrophy in the ever-ART multivariable models.

4.3.5 Multivariable models including type of ART regimen

In these models in which type of ART regimen (e.g. PI-based HAART, NNRTI-based HAART, triple therapy, mono-therapy) was included, there was adjustment for age, duration of ART, ethnicity and maximum CDC clinical stage. Triple class therapy was the only type of regimen associated with body fat alterations outcomes, with a significant increased risk of any fat alterations (AOR: 5.41, 95% CI: 1.21, 24.13, $p = 0.027$) lipohypertrophy (AOR: 4.06, 95% CI: 1.12, 14.75, $p = 0.033$), lipoatrophy (AOR: 8.32, 95% CI: 1.73, 40.07, $p = 0.008$), and combined lipohypertrophy and lipoatrophy (AOR: 6.30, 95% CI: 1.36, 29.11, $p = 0.018$): log adjusted AOR are illustrated in Figure 4-8. Adjusted odds ratios for triple class therapy were larger than the corresponding unadjusted odds ratios (Table D-3 in Appendix D).

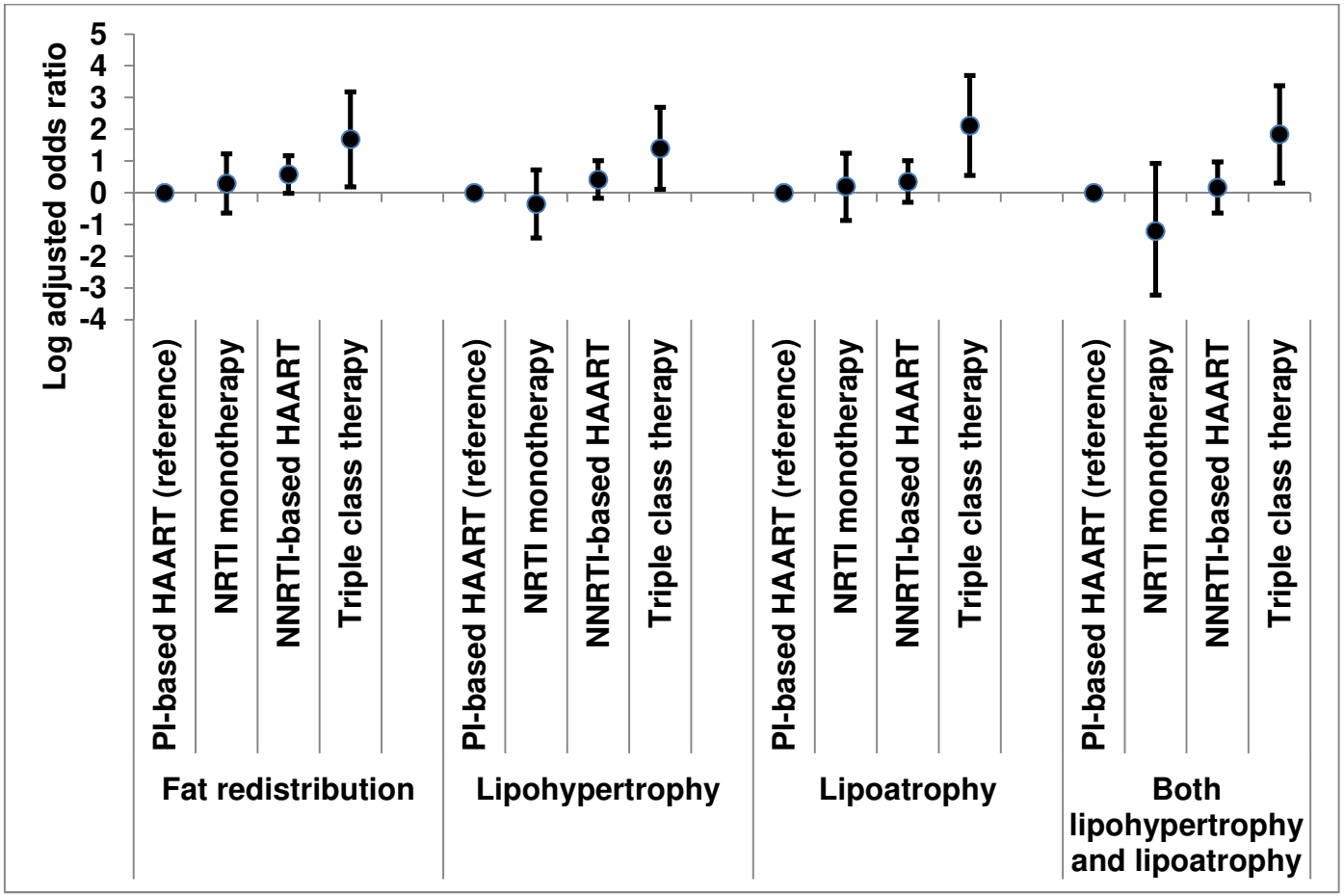
Age was associated with a significant 8-16% increased risk of each outcome per year ($p \leq 0.044$), while White ethnicity was significantly associated with an increased risk of body fat alterations (AOR: 3.09, 95% CI: 1.47, 6.48, $p = 0.003$), and lipoatrophy (AOR: 4.80, 95% CI: 1.84, 12.54, $p = 0.001$). This was in contrast to the previous multivariable models where age

was not a significant risk factor, and White ethnicity was associated an increased risk of all outcomes except lipohypertrophy. As with the final stepwise multivariable models, maximum CDC-defined clinical state was a significant positive risk factor for both any fat alterations, and lipohypertrophy.

4.3.6 Investigation of puberty as a risk factor

As discussed in Section 4.1, Tanner score was not included in the backward stepwise logistic modelling to avoid over-controlling for puberty, given that age was included in all models, and sex was included in the stepwise process. However, sex was not significant in any of the final multivariable models. In order to investigate the role of puberty further, Tanner score was included in the previously constructed final models modelling specific ART drugs at recruitment. Further models were then fitted including an interaction term between sex and age, again using the previously fitted multivariable drugs for current ART. In all these models, the additional terms were not statistically significant (Table D-6 and Table D-7 in Appendix D).

Figure 4-8 Multivariable models for fat alterations outcomes using type of antiretroviral therapy as an explanatory variable



Log adjusted odds ratio displayed for clarity. All models adjusted for age and maximum duration of ART use at recruitment, ethnicity and maximum CDC-defined clinical stage. Fat redistribution: *n* = 329, lipoatrophy *n* = 327, lipohypertrophy: *n* = 328, and combined lipoatrophy and lipohypertrophy: *n* = 329

4.4 Key points

- Prevalence of body fat alteration outcomes were significantly higher in older adolescents compared to younger children, and in White subjects compared to Black subjects. Prevalence was also higher in subjects who were undergoing or had completed puberty, had experienced symptomatic HIV disease, and among hepatitis-C co-infected individuals: these were not statistically significant for all outcomes.

- Current use of NNRTI was associated with an independent significant increased risk of all fat alterations outcomes: the size of this effect was similar for all outcomes (2-fold), but increased to 8-fold risk for the combined phenotype (Table 4-7).

Table 4-7: Summary of risk factors for fat alterations outcomes across all multivariable models.

	Fat alterations	Lipohypertrophy	Lipoatrophy	Combined lipohypertrophy and lipoatrophy
Age	↑	↑	↑	↑
Maximum duration of ART				↑
Gender		↑		
White ethnicity	↑		↑	↑
Maximum CDC clinical condition	↑	↑		
Nadir CDC immune-suppression			↓	
BMI	↑	↑		↑
NNRTI*	↑	↑	↑	↑
PI*				↑
Stavudine*	↑		↑	
Zidovudine*				↓
Lamivudine*				↓
Didanosine*	↓			
Efavirenz*	↑			
Triple class therapy*	↑	↑	↑	↑

Only factors that were statistically significant in at least one individual model are shown. Arrows indicate direction of association. *Current-use at the time of recruitment

- Although current PI use was a significant and independent risk factor for increased concurrent lipoatrophy and lipohypertrophy, individual PI drugs were not significant in any model.

- Recruitment stavudine and efavirenz were associated with an independent significant 2-5 fold increased risk of all body fat alterations outcomes

- Zidovudine and lamivudine use at recruitment were associated with a 54-67% decreased risk of concurrent lipohypertrophy and lipoatrophy.

- Current use of any specific ART drug was not significantly associated with lipohypertrophy, but ever use of stavudine and efavirenz were significant risk factors in multivariable models.

- Ever use of stavudine, efavirenz, didanosine, ritonavir booster and indinavir were independent significant risk factors for specific body fat alterations: no ever-use of ART was associated with a significant decreased risk of body fat alterations.

- Triple class therapy was associated with a 4-8 fold increased risk of all outcomes compared to PI-based HAART in multivariable analyses.

- White ethnicity and maximum clinical status were consistently associated with a significant and independent increased risk of all types of body fat abnormality

5. Metabolic abnormality and lipodystrophy syndrome at recruitment

5.1 Objectives

The main objectives of this chapter were to identify risk factors associated with prevalent metabolic abnormality and lipodystrophy syndrome at recruitment. Specific objectives were to:

- Investigate specific metabolic abnormality outcomes: (i) any metabolic abnormality, (ii) fasting hypertriglyceridemia, (iii) hypercholesterolemia, and (iv) both hypercholesterolemia and fasting hypertriglyceridemia occurring together, by estimating their prevalence

- Identify risk factors for each outcome by fitting univariable regression models.

- Determine the final multivariable model for each outcome using categories of antiretroviral therapy at recruitment as explanatory variables and to refit this in order to explore the effects of inclusion of specific drug exposure, or of ART regimen type (e.g. PI-based HAART, triple class) in place of ART class.

- Estimate the prevalence of lipodystrophy syndrome (encompassing both/either body fat alterations and metabolic abnormality), and identify risk factors in the form of individual ART drugs, classes of ART, and ART regimen, as above.

5.2 Specific methods

Methods are as described in the previous chapter (Chapter 4): this was a cross-sectional analysis using data collected at recruitment (*n* = 428). However, the outcome measures investigated here are: any metabolic abnormality, fasting hypertriglyceridemia, hypercholesterolemia, both hypercholesterolemia and fasting hypertriglyceridemia occurring together, and LS, as summarized in Box 5-1.

Box 5-1: Core outcomes investigated in this chapter

Any metabolic abnormality

Any fasting hypertriglyceridemia

Any hypercholesterolemia

Both hypercholesterolemia and fasting hypertriglyceridemia occurring together

Any lipodystrophy syndrome

Definitions of metabolic abnormality, according to serum thresholds by gender and age are provided in Table B.1 and Table B.2 in Appendix B. Lipodystrophy syndrome is defined by the occurrence of at least one the symptoms presented in Box 2 1 and Box 2 2 in Chapter 2.

ART explanatory variables are outlined in Table 2-1 in Section 2.8.6 in Chapter 2.

5.2.1 Estimation of prevalence

Prevalences of each outcome by potential risk factors (e.g. ethnicity, specific ART drug, etc.) were estimated with respect to the numbers of subjects with complete data for that factor from the total study population.

5.2.2 Univariable and multivariable regression modelling

Risk factors were identified by fitting univariable and multivariable logistic regression models using the methods described in Section 2.8.6. As previously, three groups of multivariable models were fitted: (i) models for the outcomes listed in Box 5-1 using classes of ART drugs as covariates, together with non-treatment related factors, (ii) models fitted using specific ART drugs and non-treatment related factors as explanatory variables, and (iii) models using type of ART regimen as an explanatory variable. Classes of ART and specific ART models were fitted using backward stepwise covariate selection, while ART regimen models were intuitively adjusted for other covariate.

Sensitivity analyses were conducted by re-fitting the final models including specific ART drugs as explanatory variables using moderate/severe metabolic abnormality, fasting hypertriglyceridemia, and hypercholesterolemia as outcome measures. The threshold for

moderate/severe metabolic abnormalities was designated as 20% above the original age- and gender-defined limits (outlined in Table B-1 and Table B-2 in Appendix B). Results are shown in Table E-5 in Appendix E.

Further sensitivity analyses were conducted by repeating the stepwise covariate selection but using ever-use of specific ART drugs (in place of current-use at the time of recruitment). Results of sensitivity analyses of multivariable models, and models using ever-use of ART as explanatory variables are included in Table E-4 in Appendix E.

A final set of sensitivity analyses were conducted by repeating the covariate selection procedure using a threshold of 10% statistical significance: these are illustrated in Appendix E.

Multivariable logistic regression models examining the role of ART regimen (i.e. PI-based HAART, NNRTI-based HAART, NRTI-monotherapy, and triple class therapy) on metabolic outcomes were not determined using stepwise variable selection, but only included covariates chosen *a priori*. These were age, maximum duration of ART and also detectable viral load at recruitment. Detectable viral load was included in these models as it was frequently statistically significant in the fitted univariable and multivariable models for metabolic abnormality, and furthermore reflect drug adherence.

5.2.3 Metabolic abnormality outcomes and glucose intolerance

Although glucose intolerance was included in the definition of metabolic abnormality (Box 2-2 in Chapter 2), only 7 subjects presented with this condition at recruitment: thus additional analyses investigating associations with participants' characteristics, and risk factors could not be conducted for glucose intolerance.

5.2.4 Investigation of lipodystrophy syndrome and antiretroviral regimen

Multivariable logistic regression models using type of ART regimen as an explanatory variable and LS as the response variable also included age and maximum duration of ART at recruitment, ethnicity and maximum CDC-defined clinical status. These covariates were chosen as they were identified as being statistically significant risk factors in several of the specific/categorical ART models.

5.3 Results

5.3.1 Prevalence of metabolic abnormality outcomes by subject characteristics

Table 5-1 summarizes the distribution of metabolic abnormality at recruitment by subject characteristic. No statistically significant differences were seen for any metabolic abnormality outcomes by sex. However, significant ($p < 0.05$) differences in metabolic abnormality prevalence were seen between age groups, with a greater proportion of 2-6 year olds having an abnormality compared to 7-11 year olds, and a greater proportion of 7-11 year olds having abnormal results compared to 12-18 years, for most outcomes. Across all outcomes, subjects of White ethnicity had the highest prevalence of abnormality, but there were significant differences in prevalence for only two outcomes: any metabolic abnormality (30% versus 18% for White versus Black subjects) and fasting hypertriglyceridemia (21% versus 10%). Regarding puberty, the highest prevalence of metabolic abnormality outcomes was seen in subjects at Tanner stage I. This was followed by stage V then stages II-IV in the case of any metabolic abnormality, with the converse being seen in fasting hypertriglyceridemia and combined hyperlipidemia ($p < 0.01$ for both outcomes). While a greater percentage of subjects who were not co-infected with hepatitis-C had metabolic abnormality compared to co-infected subjects, these differences were not significant.

There were no significant differences in prevalence of metabolic abnormality across maximum or recruitment CDC clinical status, or nadir or recruitment immune status. However, for most outcomes, subjects with current/previous immunosuppression or clinical symptoms had lower prevalence compared to subjects with no evidence of immunosuppression or clinical symptoms. Prevalence of all outcomes was higher in subjects with undetectable viral load: these differences were significant for any metabolic abnormality (30% vs. 20%: $p = 0.016$), hypercholesterolemia (19% vs. 6%: $p < 0.001$), or combined hypercholesterolemia and fasting hypertriglyceridemia (7% vs. 2%, $p = 0.020$).

Prevalence of any metabolic abnormality, fasting hypertriglyceridemia and the combination of hypercholesterolemia and fasting hypertriglyceridemia occurring together was greater among subjects on NRTI compared to those not on this class of ART (Table 5-1), but these differences were non-significant. However, since all but 2 subjects on ART at recruitment were on NRTI ($n/N = 362/364$), these non-significant associations are analogous to the association between these outcomes and any current ART use. Indeed, following exclusion of subjects who were ART naïve at recruitment, of the 2 subjects on NRTI at recruitment only 1 had metabolic abnormality: the differences in prevalence were significant ($p \leq 0.032$) for hypercholesterolemia, and for combined hypercholesterolemia with fasting hypertriglyceridemia (Table E-1 in Appendix E). Prevalence of all outcomes was non-significantly higher in subjects on NNRTI compared to those non on NNRTI: this is contrast to body fat alterations where prevalence was higher in subjects not currently on NNRTI.

Table 5-1: Prevalences of metabolic abnormality at recruitment (*N* = 428)

		Metabolic abnormality		Fasting hypertriglyceridemia		Hypercholesterolemia		Combined fasting hypertriglyceridemia, and hypercholesterolemia	
		%	*n/N*	*%*	*n/N*	*%*	*n/N*	*%*	*n/N*
Demographic factors									
Sex	**Male**	28	54/195	20	40/196	13	26/199	7	13/198
	Female	24	50/209	15	31/211	12	26/213	4	9/210
Age (years)	**2-6**	37***	43	26*	11/43	26***	11/43	14***	6/43
	7-11	37***	42/115	23*	26/115	24***	28/116	10***	12/116
	12-18	19***	50/257	13*	34/260	6***	17/264	2***	4/260
Ethnicity	**Black**	18*	18/102	10*	10/105	9	10/107	3	3/103
	White	30*	83/279	21*	58/279	15	14/282	6	18/282
	Other	18*	3/17	12*	2/17	0	0/17	0	0/17
Country of residence	**Italy**	26	70/270	20	269	10***	27/273	4*	11/273
	Belgium	20	16/80	10	83	11***	9/84	4*	3/80
	Poland	34	22/65	15	66	30***	20/66	12*	8/66

		Metabolic abnormality		Fasting hypertriglyceridemia		Hypercholesterolemia		Combined fasting hypertriglyceridemia, and hypercholesterolemia	
		%	*n/N*	*%*	*n/N*	*%*	*n/N*	*%*	*n/N*
Infection factors									
CDC immune stage at recruitment	**Stage 1**	28	90/316	18	56/318	15	48/322	5	17/320
	Stage 2	19	16/83	17	14/83	8	7/84	6	5/83
	Stage 3	13	2/16	6	1/17	6	1/17	0	0/16
Nadir CDC immune stage	**Stage 1**	30	48/158	18	29/158	18	28/159	7	11/158
	Stage 2	23	40/174	17	30/175	10	17/178	4	7/177
	Stage 3	24	20/83	14	12/85	13	11/86	5	4/84
CDC clinical stage at recruitment	**N+A**	26	88/340	17	59/342	13	44/344	5	18/342
	B	20	4/20	15	3/20	5	1/21	0	0/21
	C	22	2/9	11	1/9	18	2/11	9	1/11
Maximum CDC clinical stage	**N+A**	26	43/168	18	30/169	14	23/170	6	11/170
	B	28	39/140	17	24/141	12	17/143	3	4/140
	C	24	20/84	16	14/85	14	12/87	7	6/86
Viral load (copies/ml)	**≤50**	30*	73/241	23	13/57	19***	46/244	7*	4/175
	>50	20*	34/172	16	57/359	6***	10/177	2*	4/175

		Metabolic abnormality		Fasting hypertriglyceridemia		Hypercholesterolemia		Combined fasting hypertriglyceridemia, and hypercholesterolemia	
		%	*n/N*	*%*	*n/N*	*%*	*n/N*	*%*	*n/N*
Other factors									
Hepatitis C co-infection	**Uninfected**	27	104/381	18	68/384	14	54/389	5	21/385
	Infected	12	3/25	8	2/25	4	1/25	0	0/25
Tanner score for puberty	**I**	40***	53/134	26**	35/134	26***	35/135	13***	17/135
	II-IV	19***	142	13**	19/141	7***	101/145	2***	3/144
	V	20***	18/91	10**	9/94	9***	8/94	1***	1/92
ART naïve	**Not naïve**	26	102/389	17	67/391	13	52/395	14	55/394
	Naïve	23	6/26	15	4/27	14	4/28	4	1/28
NRTI									
Any NRTI	**Not current**	26	7/27	18	5/28	17	5/30	10	3/30
	Current	27	96/356	18	63/358	13	48/361	5	18/358
NNRTI									
Any NNRTI	**Not current**	29	73/254	20	51/256	15	38/259	7	17/257
	Current	23	30/129	13	17/130	11	15/132	3	4/131

		Metabolic abnormality		Fasting hypertriglyceridemia		Hypercholesterolemia		Combined fasting hypertriglyceridemia, and hypercholesterolemia	
		%	*n/N*	*%*	*n/N*	*%*	*n/N*	*%*	*n/N*
PI									
Any PI	**Not current**	19	32/170	10***	18/172	10*	17/174	2*	4/173
	Current	33	71213	23***	50/214	1/7*	36/217	8*	17/215
Atazanavir	**Not current**	28**	103/366	18	68/369	14	53/374	6	21/371
	Current	0*	0/17	0	0/17	0	0/17	0	0/17
Ritonavir booster	**Not current**	17***	33/189	10***	19/192	9**	17/195	2**	17/193
	Current	36***	70/194	26***	49/194	18**	36/196	9**	17/195
Type of drug regimen at recruitment	**PI-based HAART**	33**	65/200	23**	46/201	17	34/204	8	16/202
	NRTI immunotherapy	10**	3/29	6**	2/29	3	1/29	0	0/29
	NNRTI-based HAART	20**	23/115	10**	12/116	10	12/117	2	2/116
	Triple class	46**	6/13	31	4/13	15	2/13	8	1/13

Association between participant characteristic and metabolic abnormality (prevalence) investigated using χ^2 test: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$. CDC-defined immune status - stage 1: no immunosuppression, stage 2: moderate immunosuppression, stage 3: severe immunosuppression. CDC-defined clinical status - N + A: no symptoms/asymptomatic, B: moderate symptoms, C: severe symptoms.

As with body fat alterations outcomes, prevalence of each outcome was greater in subjects on PI at recruitment compared to those not, with prevalence of any metabolic activity reaching 30% (Table 5-1). However, in contrast to current use of PIs, there was no significant difference in any metabolic abnormality outcome between subjects who had ever used these drugs compared to those who had never used PIs (Table 5-2). Indeed, only ever-use of NNRTI had a significant association with metabolic outcomes: prevalence of both any metabolic abnormality and combined hypercholesterolemia with fasting hypertriglyceridemia were higher in subjects who had never been on NNRTI compared to those who had ever used NNRTI ($p < 0.05$). Interestingly, prevalence of all body fat alterations outcomes was significantly higher in subjects who had ever been on NNRTI compared to those who had never been on this class of ART.

Table 5-2: Prevalence of metabolic abnormality at recruitment by ever use of categories of antiretroviral therapy

		Metabolic abnormality		Fasting hyper-triglyceridemia		Hyper-cholesterolemia		Combined fasting hypertriglyceridemia, and hyper-cholesterolemia	
		%	*n/N*	*%*	*n/N*	*%*	*n/N*	*%*	*n/N*
NRTI	**Never use**	23.1	6/26	14.8	4/27	14.3	4/28	7.1	2/28
	Ever use	26.2	102/389	17.1	67/391	13.2	52/395	5.1	20/391
NNRTI	**Never use**	27.3*	48/176	18.5	33/178	16.8	30/179	8.5*	15/177
	Ever use	25.1*	60/239	15.8	38/240	10.7	26/244	2.9*	7/242
PI	**Never use**	17.2	16/93	12.6	12/95	7.3	7/96	3.2	3/95
	Ever use	28.6	92/322	18.3	59/323	15.0	49/327	5.9	19/324
ART naïve	**Treated**	27.2	97/357	17.8	64/359	13.5	49/363	5.3	19/360
	Naïve	23.1	6/26	14.8	4/27	14.3	4/28	7.1	2/28

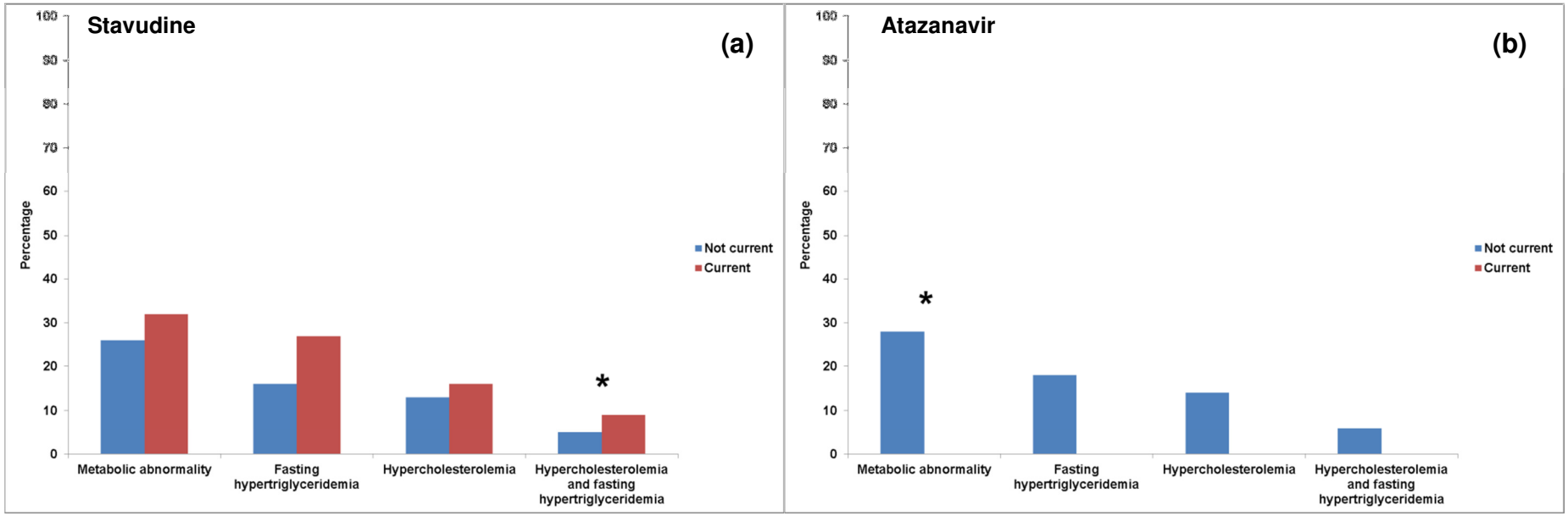
Association investigated using χ^2 test: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

The only specific NRTI to show a significant association with metabolic outcomes was stavudine, with 9% of subjects on this drug having combined hypercholesterolemia with hypertriglyceridemia compared to 5% not on the drug ($p = 0.025$) as illustrated in Figure 5-1a. Indeed, subjects who were currently on stavudine also had a significantly higher prevalence of any body fat alterations and lipoatrophy (Chapter 4).

Five PI drugs showed a significant association with prevalence of metabolic abnormality. None of the 17 subjects on atazanavir at recruitment had fasting hypertriglyceridemia (Table 5-1), while the prevalence was 18% in subjects who were not on the drug ($p = 0.011$) as illustrated in Figure 5-1b. Similarly, the prevalence of fasting hypertriglyceridemia among subjects currently on tipranavir was higher (33%) compared to those not on the drug (17%) as illustrated in Figure 5-2b, with this difference reaching statistical significance ($p = 0.047$). Subjects who were currently on ritonavir booster at recruitment had a higher prevalence of each outcome ($p < 0.01$) as illustrated Figure 5-3. Unlike body fat alterations outcomes there were no significant differences in metabolic abnormality outcomes with current/not current use of either categorical, or specific, NNRTI (Table 5-1) .

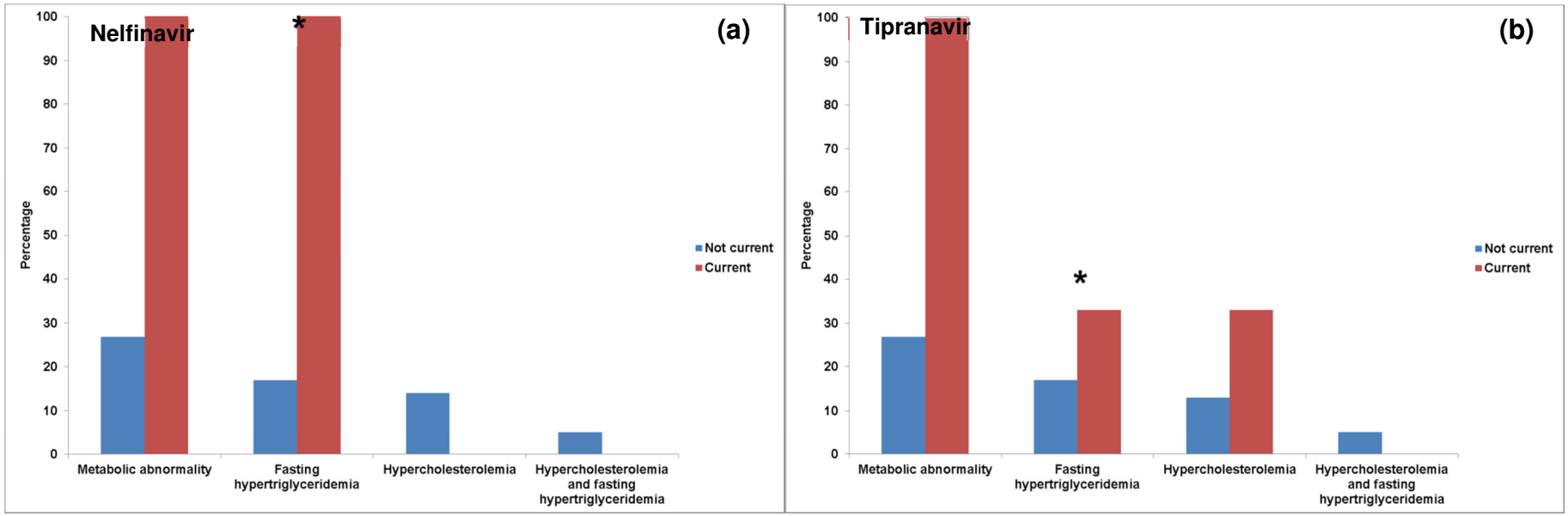
A similar relationship between types of ART across each outcome was seen: the highest prevalence was seen in subjects on triple class therapy, followed by PI-based HAART, NNRTI-based HAART and finally NRTI mono-therapy (Figure 5-4): this pattern was seen with body fat alteration outcomes (Chapter 4). These differences were significant ($p < 0.01$) for any metabolic abnormality and fasting hypertriglyceridemia; for both outcomes, the prevalence exceeded 30% in subjects on triple class therapy at recruitment.

Figure 5-1: Prevalence of metabolic abnormality outcomes by (a) stavudine, and (b) atazanavir



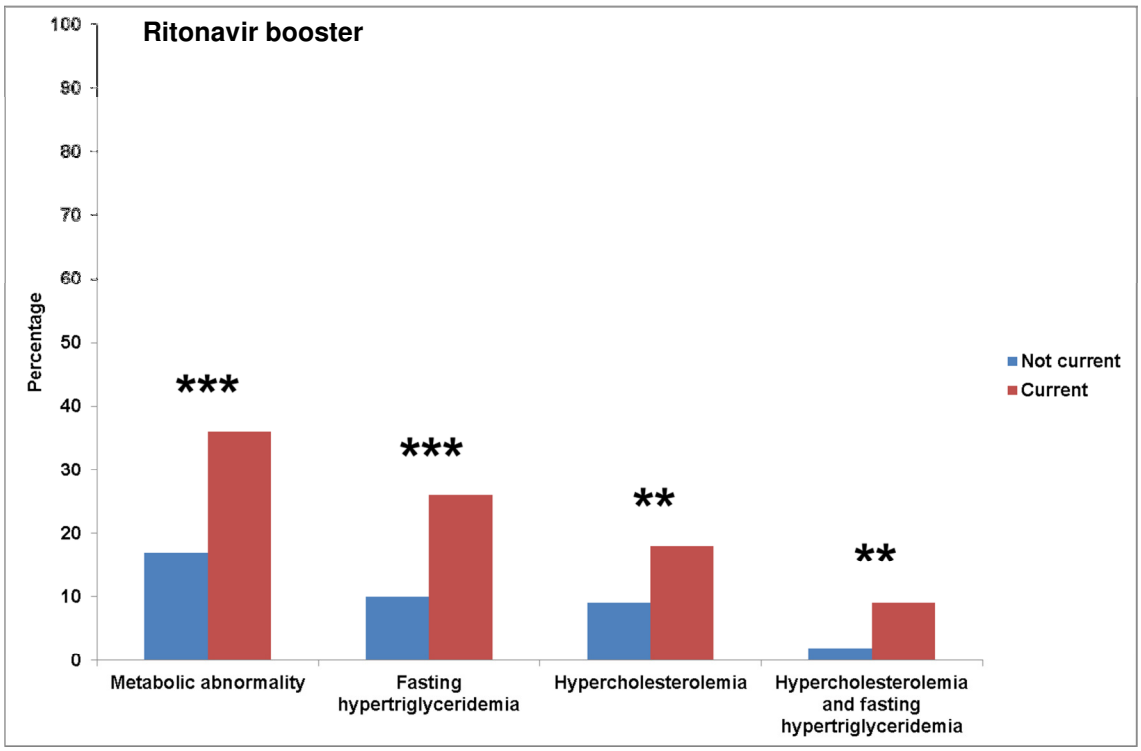
Association investigated using χ^2 test: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

Figure 5-2: Prevalence of metabolic abnormality by (a) nelfinavir, and (b) tipranavir



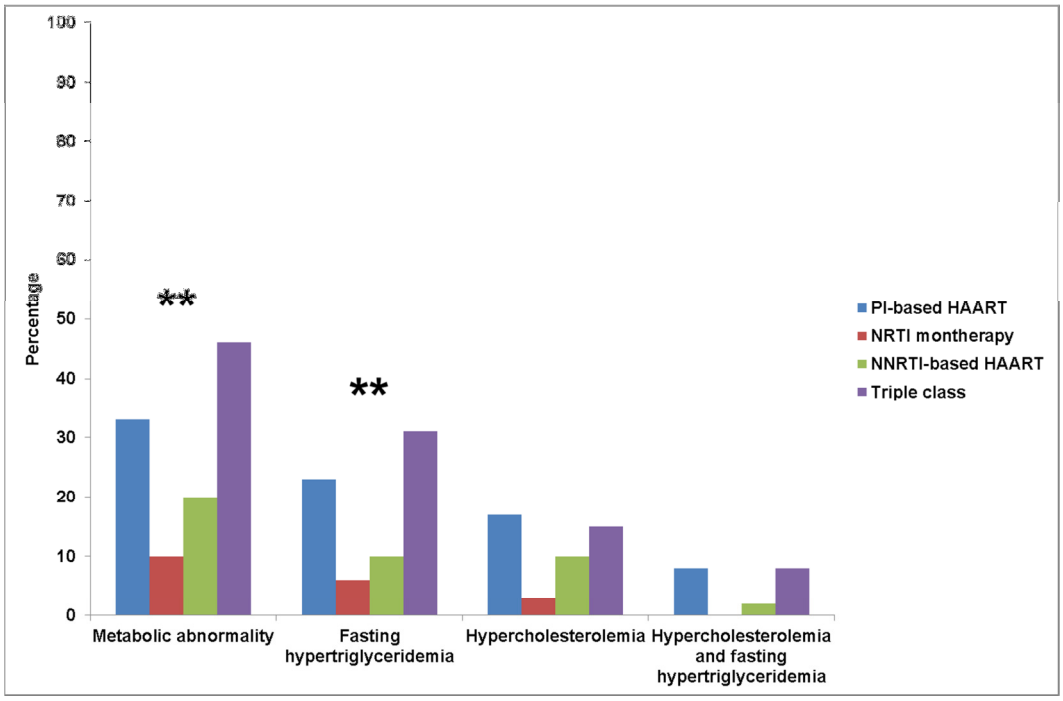
Association investigated using χ^2 test: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

Figure 5-3: Prevalence of metabolic abnormality by ritonavir booster



Association investigated using χ^2 test: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

Figure 5-4: Prevalence of metabolic abnormality outcomes at recruitment by antiretroviral therapy regimen



Association investigated using χ^2 test: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

5.3.2 Univariable analyses of factors associated with metabolic abnormality

In univariable analysis, White ethnicity was seen to be significantly ($p = 0.019$) associated with a 2-fold increased risk of any metabolic abnormality compared to Black ethnicity (Table E-2 in Appendix E). While female sex was associated with a decreased risk of all outcomes, this was not statistically significant. A significant reduced risk of all outcomes was seen in subjects aged 12-18 years compared to subjects aged 2-6 years old: the reduction in risk ranged from 56% for fasting hypertriglyceridemia, to 90% for the combined phenotype. Furthermore, subjects who were undergoing puberty (Tanner II-IV) had a significant ($p < 0.01$) 56-85% reduced risk of all metabolic outcomes compared to pre-pubescent subjects (Tanner I), and subjects who had completed puberty (Tanner V) had a significant ($p < 0.05$) 62-92% reduced risk compared to pre-pubescent subjects. This is in direct contrast to body fat alterations outcomes where increasing age, and both undergoing and completion of puberty were associated with significant increased risk.

A history of moderate immunosuppression was associated with a 51% reduced risk ($p = 0.032$) of hypercholesterolemia, but no other immunosuppression or clinical disease covariate was significantly associated with metabolic abnormality. However, detectable viral load, was associated with a reduced risk of any metabolic abnormality of over 60% ($p = 0.001$).

While both recruitment NNRTIs and NRTIs had been shown to be significantly associated with body fat alterations, no statistically significant associations between these drugs and metabolic abnormality was seen in univariable models. Use of any PI at recruitment was associated with increased risk of any metabolic abnormality (OR: 2.16, 95% CI 1.34, 3.48), fasting hypertriglyceridemia (OR: 2.61, 95% CI: 1.46, 4.67), hypercholesterolemia (OR: 1.84, 95% CI: 0.99, 3.40), and the combined phenotype (OR: 3.63, 95% CI: 1.20, 10.99). Furthermore, ritonavir booster was a risk factor for all metabolic outcomes, associated with a 2-5 fold significant increased risk ($p < 0.01$). Neither current use of PI, nor current use of any specific PI drug was a significant risk factor for any body fat alterations outcome (Chapter 4). NRTI-monotherapy was associated with a 76% reduced risk ($p = 0.023$), and NNRTI-based HAART a 48% reduced risk ($p = 0.018$) of any metabolic abnormality (compared to PI-based HAART). Similarly, triple class therapy was associated with a significant 80% reduced risk of concurrent hypercholesterolemia and fasting hypertriglyceridemia.

In univariable analyses with ever-use of either categorical or specific ART as explanatory variables, only use of any PI and of ritonavir booster were significant risk factors: both were associated with increased risk of metabolic abnormality outcomes ($p < 0.05$), as summarized in Table E-3 in Appendix E.

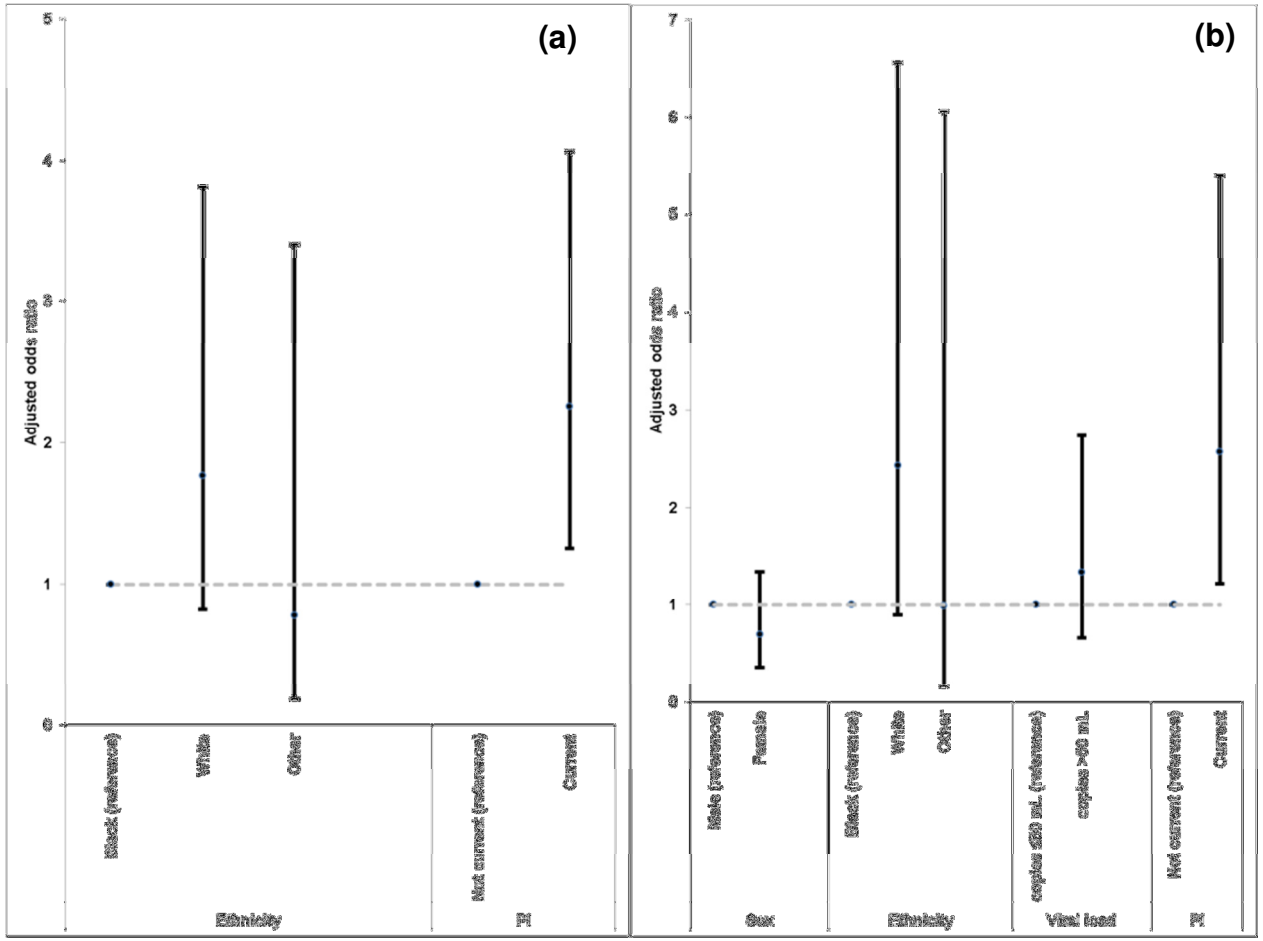
5.3.3 Multivariable models for metabolic abnormality outcomes including antiretroviral therapy class

In constructing the final multivariable models for class of ART, the models included age and maximum duration of ART at recruitment. The models are summarized in Figure 5-5 and Figure 5-6.

Current use of PI at recruitment was a significant risk factor for all outcomes: over 2-fold for any metabolic abnormality (AOR: 2.26, 95% CI: 1.25, 4.06), 2.5 fold for fasting hypertriglyceridemia (AOR: 2.57, 95% CI: 1.22, 5.40), 2-fold for hypercholesterolemia (AOR: 2.21, 95% CI: 1.07, 4.56), and over 6-fold for concurrent hypercholesterolemia and fasting hypertriglyceridemia (AOR: 6.18, 95% CI: 1.37, 37.85).

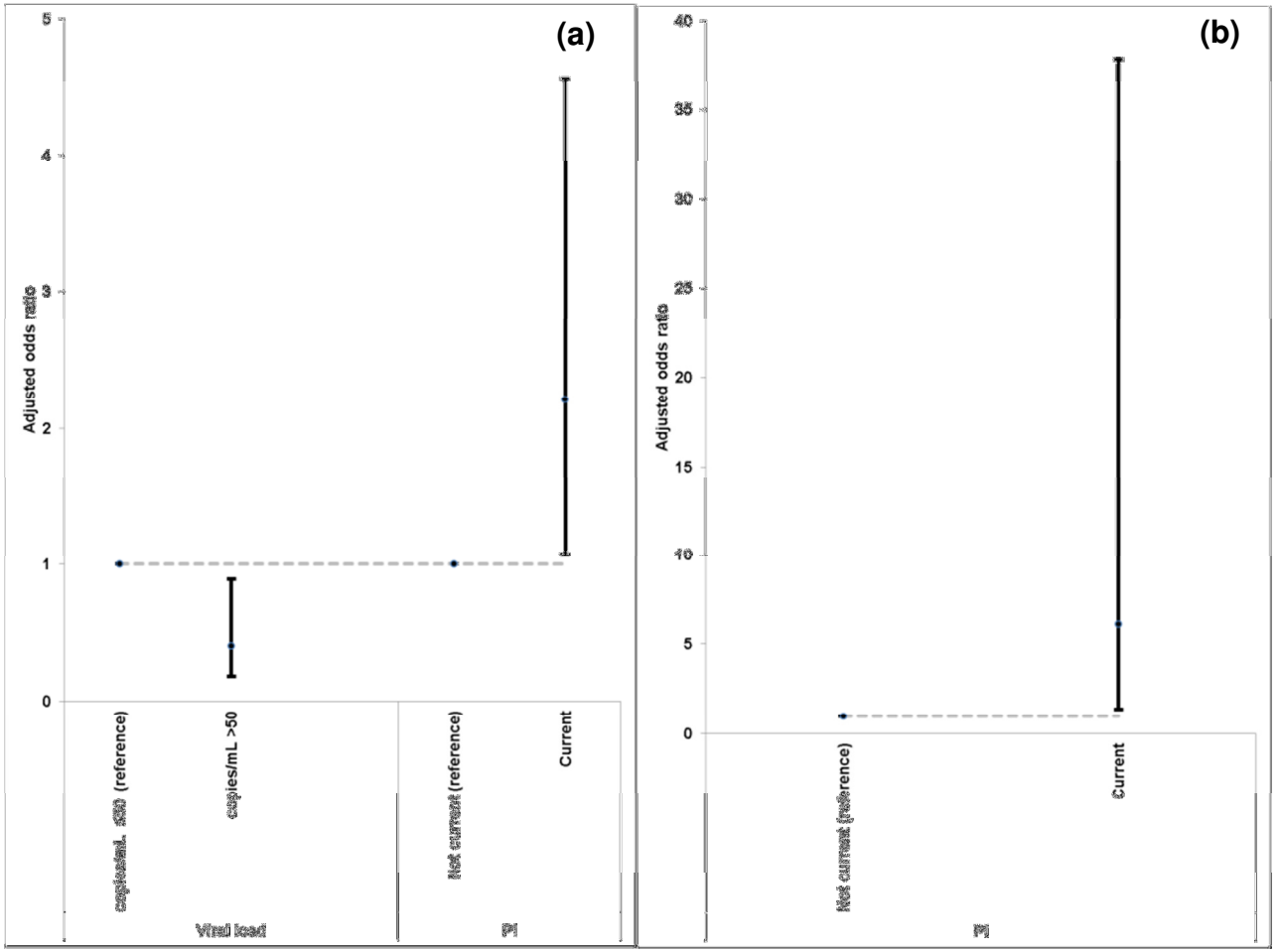
The only other significant risk factor for metabolic abnormality outcomes was detectable viral load. This was associated with a reduced risk of hypercholesterolemia (AOR: 0.40, 95% CI; 0.18, 0.89).

Figure 5-5: Final multivariable model for (a) any metabolic abnormality (*n* = 317), and (b) fasting hypertriglyceridemia (*n* = 309) using classes of antiretroviral therapy as explanatory variables



All models adjusted for age and maximum duration of ART

Figure 5-6: Final multivariable model for (a) hypercholesterolemia (*n* = 331), and (b) hypercholesterolemia and fasting hypertriglyceridemia (*n* = 329) using classes of antiretroviral therapy as explanatory variables



All models adjusted for age and maximum duration of ART

5.3.4 Multivariable models for metabolic abnormality outcomes including specific antiretroviral drugs

Metabolic abnormality

The final model for any metabolic abnormality included covariates for age and duration of ART at recruitment, and ethnicity (Table 5-3). Only one drug remained significant in the final model: use of ritonavir booster was associated with an independent almost 3-fold increased risk of metabolic abnormality (reinforcing results from the multivariable model using class of ART). At the same time, age was significantly ($p < 0.001$) associated with a 13% decrease in risk per year.

Table 5-3: Final multivariable model for metabolic abnormality using specific antiretroviral therapy drugs (*n* = 315)

		n	AOR	95% CI	*p*-value
Age	**Per year**	315	0.87	(0.81, 0.94)	<0.001
Maximum duration of ART	**Per year**	315	0.97	(0.90, 1.05)	0.483
Ethnicity	**Black**	77	1		
	White	223	1.64	(0.77, 3.49)	0.201
	Other	15	0.75	(0.17,3.25)	0.699
Ritonavir booster	**Not current**	144	1		
	Current	171	2.79	(1.56, 4.99)	0.001

Model intuitively adjusted for age and duration of ART at recruitment: Adjusted odds ratio (AOR) and 95% confidence interval (95% CI) for metabolic abnormality by participant characteristic.

Fasting hypertriglyceridemia

The final multivariable model for fasting hypertriglyceridemia risk factors included covariates for age, viral load, duration of ART, sex, and ethnicity. Ritonavir booster was associated with a significantly increased risk (AOR = 3.35), while a yearly increment of age significantly (p = 0.004) reduced risk by 12% (Table 5-4).

Table 5-4: Final multivariable model for fasting hypertriglyceridemia using specific antiretroviral drugs (*n* = 309)

		n	AOR	95% CI	*p*-value
Age	**Per year**	309	0.88	(0.80, 0.96)	0.004
Maximum duration of ART	**Per year**	309	0.99	(0.90, 1.09)	0.830
Sex	**Male**	161	1		
	Female	148	0.69	(0.36, 1.32)	0.263
Ethnicity	**Black**	79	1		
	White	215	2.20	(0.85, 6.22)	0.102
	Other	15	0.96	(0.16, 5.96)	0.969
Viral load (copies/ml)	**<50**	195	1		
	>50	114	1.30	(0.64, 2.66)	0.471
Ritonavir booster	**Not current**	141	1		
	Current	168	3.35	(1.60, 7.04)	0.001

Model intuitively adjusted for age and duration of ART at recruitment: Adjusted odds ratio (AOR) and 95% confidence interval (95% CI) for fasting hypertriglyceridemia by participant characteristic

Hypercholesterolemia

Age was associated with an 18% reduced risk per year, and detectable viral load 61% reduced risk of hypercholesterolemia ($p \leq 0.02$), as illustrated in Table 5-5. Ritonavir booster use at recruitment was associated with an almost 3-fold significant increased risk of hypercholesterolemia (AOR: 2.82, 95% CI: 1.36, 5.83).

Table 5-5: Final multivariable model for hypercholesterolemia using specific antiretroviral therapy drugs (*n* = 331)

		n	AOR	95% CI	*p*-value
Age	**Per year**	331	0.82	(0.75, 0.90)	<0.001
Maximum duration of ART	**Per year**	331	1.01	(0.91, 1.12)	0.887
Viral load (copies/ml)	**≤50**	208	1		
	>50	123	0.39	(0.17, 0.86)	0.020
Ritonavir booster	**Not current**	152	1		
	Current	179	2.82	(1.36, 5.83)	0.005

Model intuitively adjusted for age and duration of ART at recruitment: Adjusted odds ratio (AOR) and 95% confidence interval (95% CI) for hypercholesterolemia by participant characteristic.

Concurrent hypercholesterolemia and fasting hypertriglyceridemia

As with other metabolic outcome multivariable models, both age and use of ritonavir at recruitment were significant risk factors for the combined phenotype of both hypercholesterolemia and fasting hypertriglyceridemia occurring together. Current use of ritonavir was associated with a significant (p = 0.009) 7.5-fold increased risk, but this was accompanied by a very wide 95% confidence interval (Table 5-6). Age was significantly associated (p < 0.001) with a 29% reduced risk per year in the final multivariable model adjusted for maximum duration of ART at recruitment.

Table 5-6: Final multivariable model for concurrent fasting hypertriglyceridemia and hypercholesterolemia occurring together using specific antiretroviral drugs (*n* =329)

		n	AOR	95% CI	*p*-value
Age	**Per year**	329	0.71	(0.59, 0.85)	<0.001
Maximum duration of ART	**Per year**	329	1.22	(1.01, 1.48)	0.037
Ritonavir booster	**Not current**	151	1		
	Current	178	7.46	(1.66, 33.57)	0.009

Model intuitively adjusted for age and duration of ART at recruitment: Adjusted odds ratio (AOR) and 95% confidence interval (95% CI) for hypercholesterolemia by participant characteristic.

Sensitivity analyses, where the final models were refitted using moderate/severe outcomes, confirmed the results of the multivariable models: in each model current-use of ritonavir was associated with a significant and independent increase in risk. However, the magnitude of the estimated risk ranged between 4 to 5 fold (Table E-5 in Appendix E). In additional analysis where final models were ascertained using stepwise covariate selection using ever-use of specific ART as explanatory variables, ever use of ritonavir booster was the only significant ART risk factor. However, for each metabolic outcome, the significant risk associated with ever-use of ritonavir booster was lower than the risk seen for current-ritonavir booster use (Table E-4 in Appendix E). In sensitivity analyses where the threshold for inclusion of covariates in the multivariable models was raised to 10%, both PI and NNRTI use were associated with increased risk of any metabolic abnormity, while detectable viral load was significantly associated with decreased risk of hypercholesterolemia, and also of hypercholesterolemia with fasting hypertriglyceridemia occurring together (Appendix E: Table E-8). Neither NNRTI nor detectable viral load were statistically significant in the models resulting from a 5% significance threshold. With regard to models investigating specific ART drugs, efavirenz was seen to be associated with increased risk of fasting hypertriglyceridemia (in addition to ritonavir booster), as illustrated in Table E-9 in Appendix E. Other models with the 10% threshold were of similar structure to the models with the 5% threshold.

Across multivariable models for metabolic abnormality outcomes, use of PI, and specifically ritonavir booster, at recruitment was a positive independent risk factor. Similarly, age at recruitment was consistently a significant risk factor for a decrease in risk. The largest effect sizes associated with either drug use or increasing age were seen in children and adolescents with both hypercholesterolemia and fasting hypertriglyceridemia occurring together. This is in contrast to body fat alterations models (Chapter 4) where neither age nor specific PI use was associated with outcomes, but categorical NNRTI and specific NNRTI and NRTI drugs were significant risk factors. Moreover, while HIV-related factors, such as degree of immunosuppression or clinical condition, were significant risk factors for body fat alterations, they were not significant factors for metabolic abnormality.

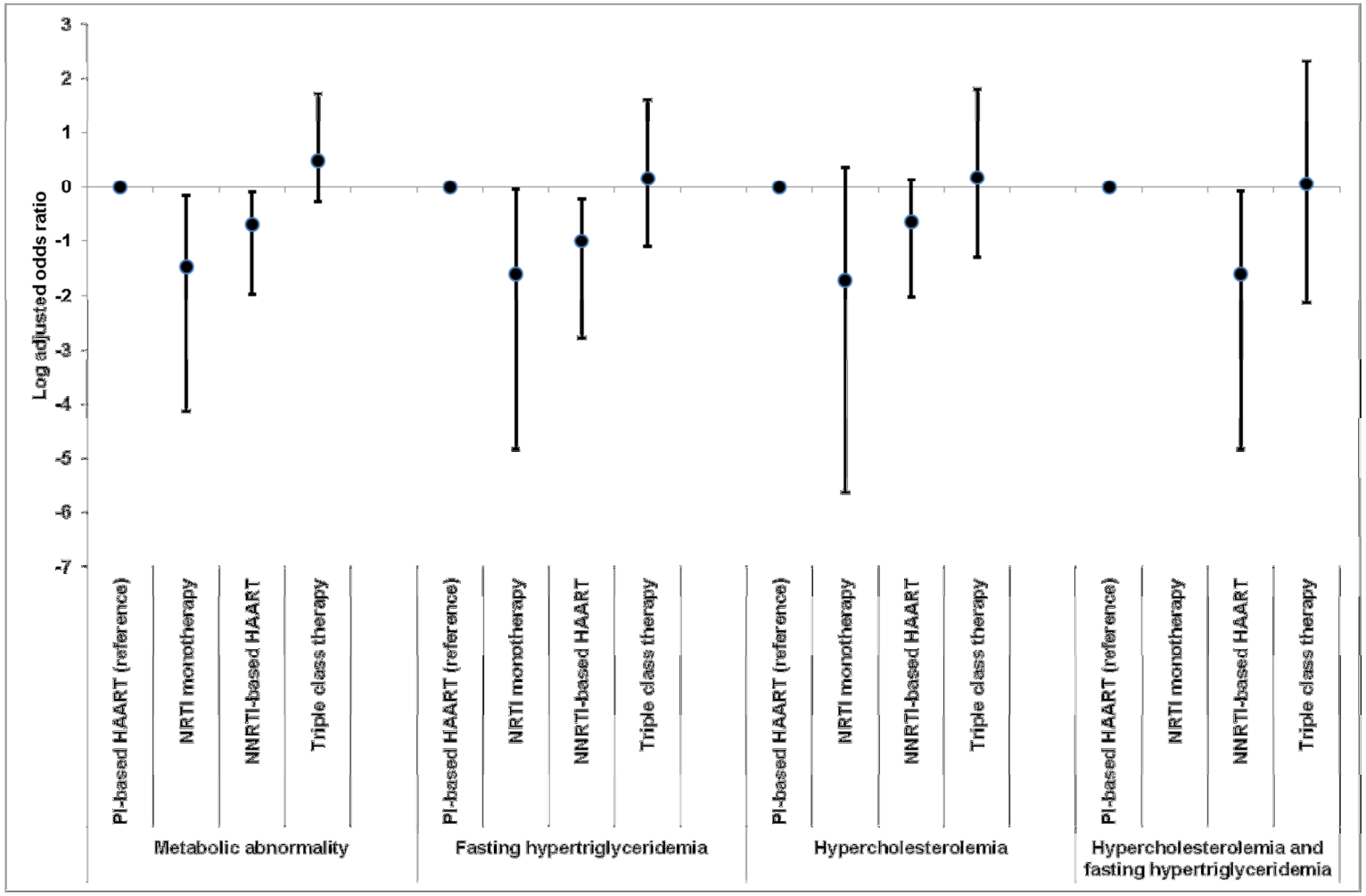
5.3.5 Multivariable models for metabolic abnormality outcomes including type of antiretroviral therapy regimen

In order to investigate the effects of type of ART regimen (i.e. PI-based HAART, NNRTI-based HAART, etc.), multivariable models adjusted for age, maximum duration of ART and viral load at recruitment were constructed. Children receiving NNRTI-based HAART at enrolment had a significant reduced risk of metabolic abnormality (AOR: 0.50, 95% CI: 0.28, 0.92), of fasting hypertriglyceridemia (AOR: 0.37, 95% CI: 0.17, 0.80), and of both hypercholesterolemia and fasting hypertriglyceridemia occurring together (AOR: 0.20, 95% CI: 0.04, 0.93), compared with those receiving PI-based HAART (Figure 5-7)

While a 47% reduced risk of hypercholesterolemia was seen with NNRTI-based HAART, this was statistically not significant (p = 0.107). The small number of children receiving NRTI mono-therapy had a significantly reduced risk of any metabolic abnormality (AOR: 0.23, 95% CI: 0.07, 0.85, p = 0.027) and of fasting hypertriglyceridemia (AOR: 0.20, 95% CI: 0.04, 0.96, p = 0.044) compared with those on -PI-based HAART.

In contrast to findings for body fat alterations where it was the only ART regimen which was statistically significant, triple class therapy was associated with a non-significant increased risk of each metabolic outcome, the large confidence interval reflecting the fact that relatively few (n = 14) subjects were on triple class therapy at recruitment.

Figure 5-7: Multivariable models for metabolic abnormality outcomes using type of antiretroviral therapy as an explanatory variable



Log adjusted odds ratio displayed for clarity. All models including terms for age, maximum duration of ART use and viral load. Metabolic abnormality: *n* = 325, fasting hypertriglyceridemia: *n* = 327, hypercholesterolemia: *n* = 331, and combined hypercholesterolemia and fasting hypertriglyceridemia: *n* = 328

5.3.6 Investigation of puberty as a risk factor for metabolic abnormality outcomes

The final multivariable models using specific antiretroviral drugs as explanatory variables were refitted with the addition of Tanner score to explore the possible effects of puberty, beyond including age and sex in the model selection process. Tanner score was statistically non-significant ($p > 0.05$) in the models for hypercholesterolemia, fasting hypertriglyceridemia, and concurrent hypercholesterolemia with fasting hypertriglyceridemia (Table E-6 in Appendix E). However, Tanner score was significant in the model for any metabolic abnormality with subjects who were undergoing puberty having a reduced risk compared with pre-pubescent subjects (AOR: 0.37, 95% CI: 0.15, 0.94, $p = 0.036$). Furthermore, although age was still associated with a reduced risk of metabolic abnormality in this model, it became non-significant. However, on the addition of an interaction term between age and sex to the original final models, no metabolic outcome model retained significant terms for either sex or the sex/age interaction (Table E-7 in Appendix E), while age remained significant only in the model for metabolic abnormality (AOR: 0.91, 95% CI: 0.82, 1.00, $p = 0.047$). This suggests that while puberty may have an independent association with the risk of any metabolic abnormality, the independent negative effect of age, also seen across metabolic outcomes, may be an important risk factor.

5.3.7 Prevalence of lipodystrophy syndrome by subject characteristics

There were no significant differences in prevalence of LS between sexes, age groups, Tanner score groups and those with/without hepatitis C infection (Table 5-7). Despite significant differences in prevalence of both body fat alterations and of metabolic outcome across age group, no significant differences were seen with LS. Similarly, while significant differences by Tanner score across metabolic abnormality outcomes, and all but one (combined phenotype) of body fat alterations outcomes, no significant differences were seen with LS.

However, as illustrated in Table 5-7, the prevalence of LS was significantly higher in subjects of White compared to Black and “Other” ethnicity. Among subjects with LS, over 80% ($n = 183$) were of White ethnicity, compared to 54.3% ($n = 95$) of subjects without LS (Figure 5-8). Indeed, prevalence of either body fat alterations or metabolic abnormalities had been seen to be significantly higher among subjects of White ethnicity compared to other subjects.

No significant differences were seen in LS prevalence between nadir immune status groups (Table 5-7). However, in common with body fat alterations, there were significant differences ($p = 0.005$) in prevalence in recruitment immune status, with prevalence highest in subjects without immunosuppression. Indeed, among subjects with LS, over 80% ($n = 193$) has a history of no immunosuppression (Figure 5-8).

Table 5-7: Prevalences of lipodystrophy syndrome at recruitment

		%	n/N	p-value
Demographic factors				
Gender	**Male**	55	108/196	0.639
	Female	57	120/209	
Age (years)	**2-6**	47	20/43	0.314
	7-11	60	69/115	
	12-18	57	146/258	
Ethnicity	**Black**	34	35/104	<0.001
	White	66	183/278	
	Other	35	6/17	
Country of residence	**Italy**	58	156/269	<0.001
	Belgium	38	31/82	
	Poland	74	48/65	
Infection factors				
CDC immune stage at recruitment	**Stage 1**	61	193/317	0.005
	Stage 2	42	35/83	
	Stage 3	44	7/16	
Nadir CDC immune stage	**Stage 1**	60	94/158	0.444
	Stage 2	53	94/174	
	Stage 3	58	49/84	
CDC clinical stage at recruitment	**N+A**	55	189/341	0.627
	B	55	11/20	
	C	40	4/10	
Maximum CDC clinical stage	**N+B**	46	78/168	<0.001
	B	66	93/141	
	C	62	52/84	
Viral load (copies/ml)	**≤50**	62	149/242	0.014
	>50	49	85/172	
Other factors				
Hepatitis C co-infection	**Uninfected**	56	215/383	
	Infected	67	16/24	0.312
Tanner score for puberty	**I**	56	75/134	
	II-IV	54	75/143	
	V	58	53/91	0.786
ART naive				
	Not naive	58	226/390	
	ART naïve	35	9/26	0.020
NRTI				
Any NRTI	**Not current**	39	11/28	
	Current	60	212/356	0.036
Stavudine	**Not current**	57	161/282	
	Current	61	66/102	0.016
NNRTI				
Any NNRTI	**Not current**	57	144/255	
	Current	61	79/129	0.0371
PI				
Any PI	**Not current**	52	87/169	
	Current	63	136/215	0.020
Ritonavir booster	**Not current**	50	94/188	
	Current	66	129/196	0.002
Type of drug therapy at recruitment				
	PI-based HAART	62	125/202	0.047
	NRTI mono-therapy	41	12/29	
	NNRTI-based HAART	58	66/114	
	Triple class	85	11/13	

Association between participant characteristic and lipodystrophy syndrome (prevalence) investigated using χ^2 test: CDC-defined immune status - stage 1: no immunosuppression, stage 2: moderate immunosuppression, stage 3: severe immunosuppression. CDC-defined clinical status - N + A: no symptoms/asymptomatic, B: moderate symptoms, C: severe symptoms.

No statistically significant ($p = 0.627$) differences were seen between LS and CDC clinical status at recruitment, but significant ($p < 0.001$) differences occurred between LS and maximum CDC clinical status: 46% were asymptomatic, 66% were at clinical stage B, and 62% were at clinical stage C (Table 5-7). Indeed, among subjects with LS, the greatest proportion of subjects had a history of moderate clinical symptoms (Figure 5-8) Significant differences with body fat alterations, but not metabolic abnormality, had previously been seen with maximum CDC clinical condition. While a similar pattern in prevalence was seen between immunosuppression groups at nadir and recruitment measurements, the pattern of LS prevalence with clinical symptoms was different between maximum and recruitment measurements.

LS prevalence was significantly ($p = 0.014$) higher among subjects with undetectable viral load, compared to subjects with detectable viral load (Table 5-7). While prevalence of both fat alterations outcomes and metabolic outcomes has been higher in subjects with undetectable viral load, these differences were only significant in the latter.

As with any body fat alterations and lipoatrophy, there was a significant ($p = 0.036$) difference in the prevalence of LS between subjects on NRTI at the time of recruitment, compared to those not, as illustrated in Figure 5-9: over 95% of subjects with LS were on NRTI at recruitment. A significant ($p = 0.020$) difference was seen in LS prevalence with current PI use: prevalence was 63% in subjects currently on PI, compared to 52% in subjects not on these drugs. Significant differences in combined lipoatrophy and lipohypertrophy had previously been seen with current NNRTI use at recruitment. Although the prevalence of LS was higher among subjects currently on NNRTI compared to those not on this class of drug (Table 5-7), this difference was not statistically significant with approximately two-thirds of both subjects with LS and those without LS currently on NNRTI on recruitment: thus only significant differences in prevalence of metabolic abnormality outcomes were seen with current PI use.

Ever-use of all class of ART, except NNRTI, was associated with an increased prevalence of LS compared to never use, and this difference was statistically significant (Table 5-8). However, as discussed previously, ever-use of NRTI is correlated to ever-use of any ART, and thus the independent prevalence of LS with NRTI-use is difficult to estimate.

Figure 5-8: Distribution of subject characteristics by lipodystrophy syndrome status at recruitment

Lipodystrophy syndrome *n* = 235, no lipodystrophy syndrome: *n* = 181

Table 5-8: Prevalence of lipodystrophy syndrome by ever-use of categories of antiretroviral therapy

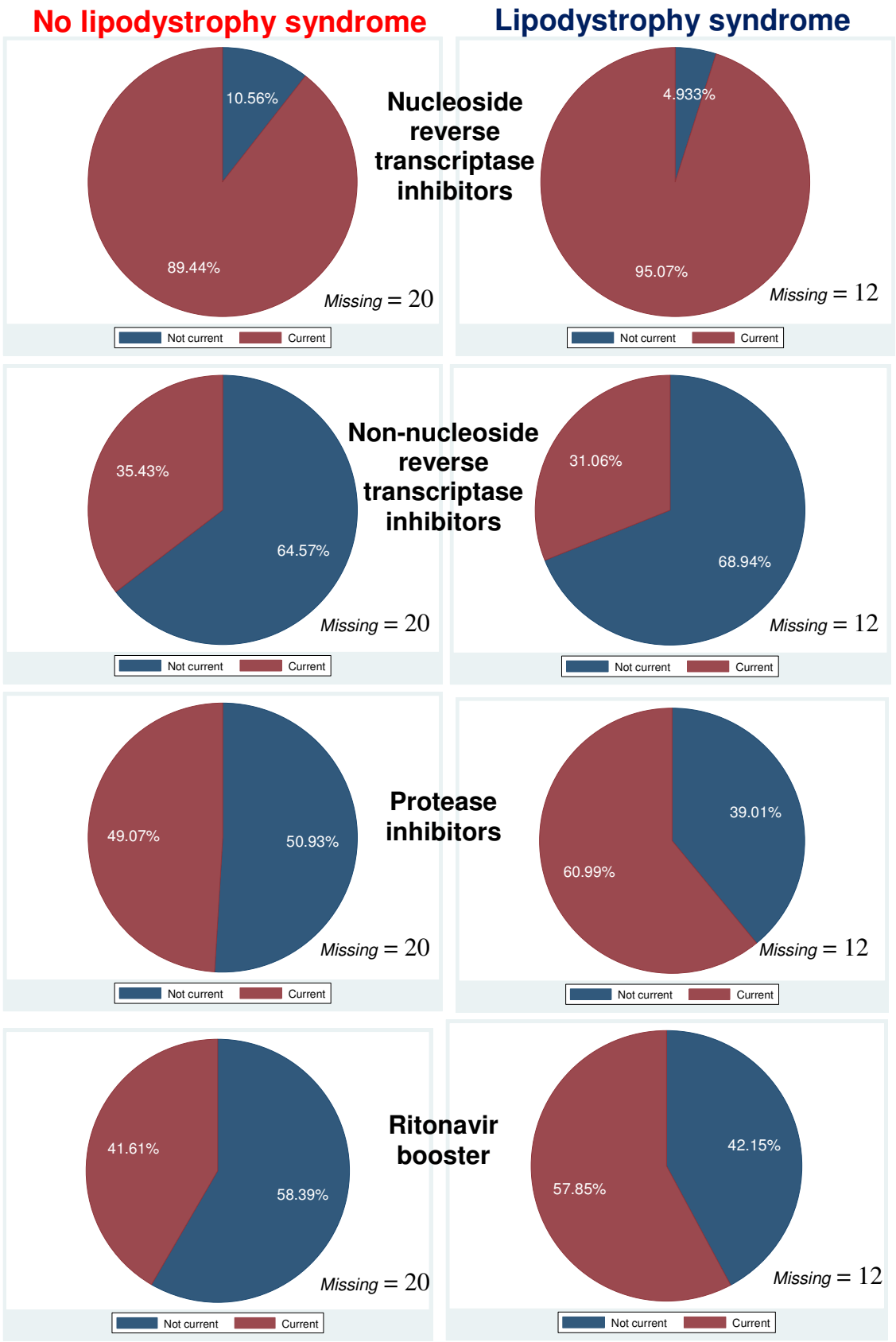
		Percentage	*n*/*N*
NRTI	**Never use**	34.6*	9/26
	Ever use	58.0*	226/390
NNRTI	**Never use**	51.4	91/177
	Ever use	60.3	144/239
PI	**Never use**	41.3***	38/92
	Ever use	60.8***	197/324
ART naïve	**Not naïve**	59.8*	214/358
	Naive	34.6*	9/26

Association between participant characteristic and lipodystrophy syndrome (prevalence) investigated using χ^2 test: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

There were significant differences ($p = 0.002$) in prevalence of LS between subjects who were currently on ritonavir booster (50%, $n = 94$) and those who were not (66%, $n = 129$): less than half of subjects with LS were on ritonavir booster at recruitment, whereas more than half of subjects without LS were on this specific ART (Figure 5-9). No significant differences were seen with other specific ART drugs.

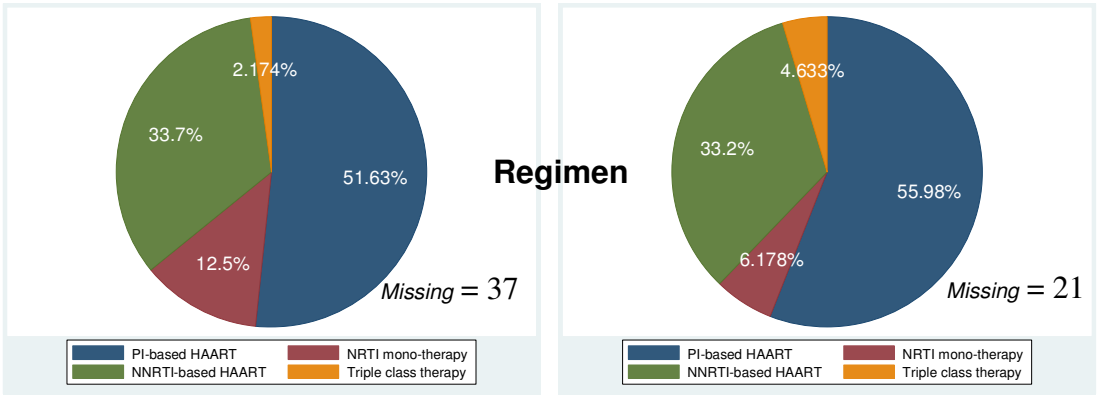
The highest prevalence of LS was seen in subjects currently on triple class therapy at the time of recruitment followed by PI-based HAART NNRTI-based HAART and finally NRTI mono-therapy (Table 5-7). Differences in prevalence between different ART combination groups were statistically significant ($p = 0.047$). Indeed the proportion of subjects on either PI-based HAART or triple class therapy was greater among subjects with LS compared to subjects without LS (Figure 5-10). However, less than 10% of the whole study population ($n = 43$) were on either NRTI mono-therapy and triple class therapy at recruitment.

Figure 5-9: Distribution of antiretroviral therapy by lipodystrophy syndrome status at recruitment.



Lipodystrophy syndrome *n* = 235, no lipodystrophy syndrome: *n* = 181. Blue: not current, red: current-use

Figure 5-10: Distribution of antiretroviral therapy regimen by lipodystrophy syndrome status at recruitment.



Lipodystrophy syndrome *n* = 235, no lipodystrophy syndrome: *n* = 181. Blue: PI-based HAART, red: NRTI-based mono-therapy, green: NNRTI-based HAART, yellow: triple class therapy

5.3.8 Univariable analyses of factors associated with lipodystrophy syndrome

There was a significant increased risk of LS associated with White compared to Black ethnicity (OR: 3.80, 95% CI: 2.36, 6.11) as illustrated in Table 5-9. Maximum CDC clinical stage was also associated with a significantly increased risk of LS compared to the asymptomatic stage: a 2-fold risk was associated with both stage B and stage C.

A significant 2-3 fold increased risk of LS was seen with current use of NRTI, and also with stavudine specifically: significant 2-4 fold increased risk of any body fat alterations or lipoatrophy had previously been seen in children on any NRTI or stavudine at recruitment. Similarly, a significant increased risk of LS was associated with use of any PI (OR: 1.62, 95% CI: 1.08, 2.44) and with ritonavir-booster (OR: 1.93, 95% CI: 1.28, 2.90) specifically at recruitment.

Significant factors associated with a reduced risk of LS were moderate CDC immune stage at recruitment, detectable viral load, and NRTI mono-therapy: reductions in risk being 53%, 39% and 57% (compared to PI-based HAART) respectively.

Table 5-9: Univariable analyses for lipodystrophy syndrome

		OR	95% CI	*p*-value
Demographic factors				
Gender	**Male**	1		
	Female	1.10	(0.74, 1.63)	0.639
Age (years)	**2-6**	1		
	7-11	1.72	(0.85, 3.49)	0.130
	12-18	1.50	(0.78, 2.87)	0.221
Ethnicity	**Black**	1		
	White	3.80	(2.36, 6.11)	<0.001
	Other	1.08	(0.37, 3.15)	0.895
Country of residence	**Italy**	1		
	Belgium	0.44	(0.26, 0.73)	0.002
	Poland	2.05	(1.12, 3.74)	0.020
Infection factors				
CDC immune stage at recruitment	**Stage 1**	1		
	Stage 2	0.47	(0.29, 0.77)	0.002
	Stage 3	0.50	(0.18, 1.38)	0.180
Nadir CDC immune stage	**Stage 1**	1		
	Stage 2	0.76	(0.49, 1.18)	0.225
	Stage 3	0.95	(0.56, 1.63)	0.861
CDC clinical stage at recruitment	**N+A**	1		
	B	0.98	(0.40, 2.43)	0.970
	C	0.54	(0.15, 1.93)	0.341
Maximum CDC clinical stage	**N+B**	1		
	B	2.24	(1.41, 3.55)	0.001
	C	1.87	(1.10, 3.20)	0.021
Viral load (copies/ml)	**≤50**	1		
	>50	0.61	(0.41, 0.91)	0.014
Other factors				
Hepatitis C co-infection	**Uninfected**	1		
	Infected	1.56	(0.65, 3.74)	0.316
Tanner score for puberty	**I**	1		
	II-IV	0.89	(0.55, 1.43)	0.631
	V	1.06	(0.62, 1.82)	0.820
NRTI				
Any NRTI	**Not current**	1		
	Current	2.28	(1.04, 5.00)	0.041
Stavudine	**Not current**	1		
	Current	2.37	(1.16, 4.84)	0.018
NNRTI				
Any NNRTI	**Not current**	1		
	Current	1.22	(0.79, 1.88)	0.371
PI				
Any PI	**Not current**	1		
	Current	1.62	(1.08, 2.44)	0.021
Ritonavir booster	**Not current**	1		
	Current	1.93	(1.28, 2.90)	0.002
Type of drug therapy at recruitment				
	PI-based HAART	1		
	NRTI mono-therapy	0.43	(0.20, 0.96)	0.039
	NNRTI-based HAART	0.85	(0.53, 1.35)	0.457
	Triple class	3.39	(0.73, 15.70)	0.119

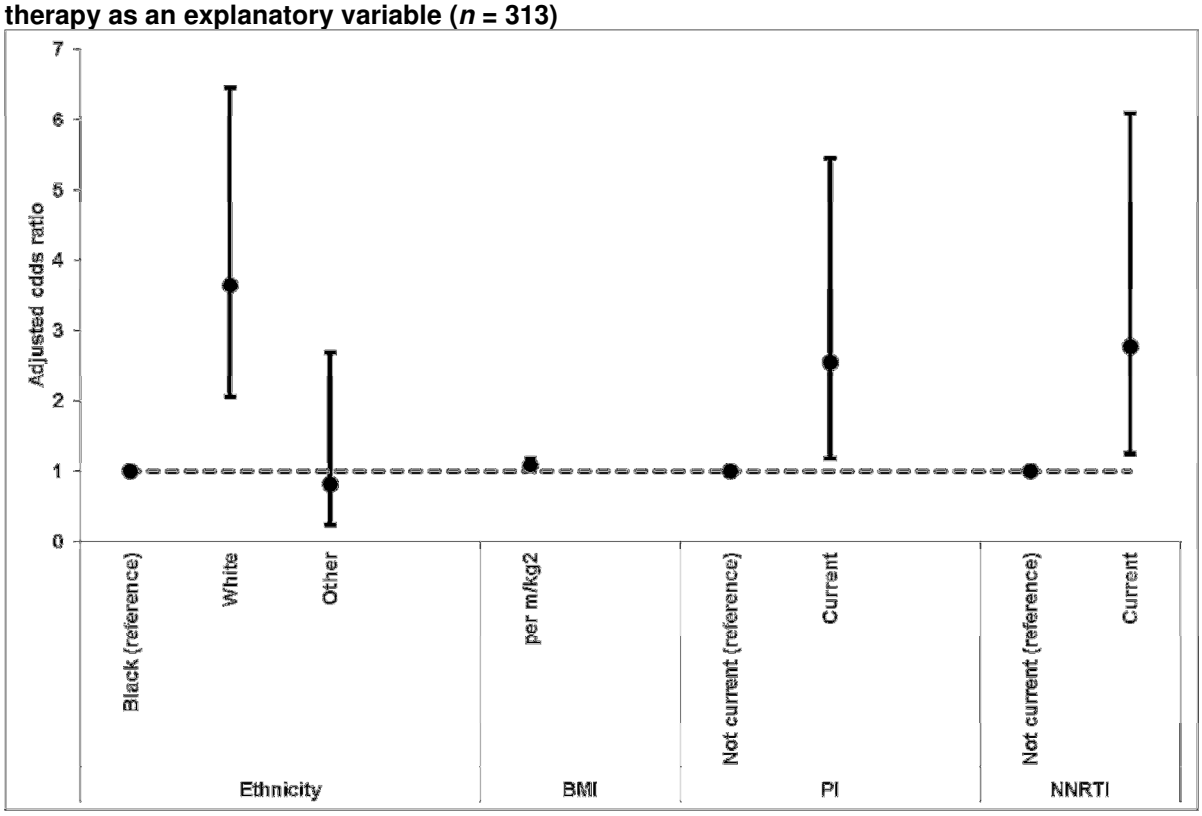
OR: odds ratio and 95% CI: 95% confidence interval

5.3.9 Multivariable models of factors associated with lipodystrophy syndrome

Final model for class of antiretroviral therapy

In the final multivariable model (adjusted for age and maximum duration of ART at recruitment) with class of ART as explanatory variables (Figure 5-11), several factors were associated with increased risk of LS: White compared to Black ethnicity (AOR: 3.65, 95% CI: 2.06, 4.46, p = 0.001), current PI (AOR: 2.56, 95% CI: 1.20, 5.45, p = 0.015), current NNRTI (AOR: 2.78, 95% CI: 1.26, 6.11, p= 0.011), and BMI per 1 unit kg/m^2 (AOR: 1.09, 95% CI: 1.00, 1.19, p = 0.049). No factors were associated with a significant reduced risk, and neither the effects of neither age nor duration of ART were statistically significant (p > 0.167).

Figure 5-11: Final multivariable model for lipodystrophy using classes of antiretroviral therapy as an explanatory variable (*n* = 313)



Model includes age and maximum duration of ART use.

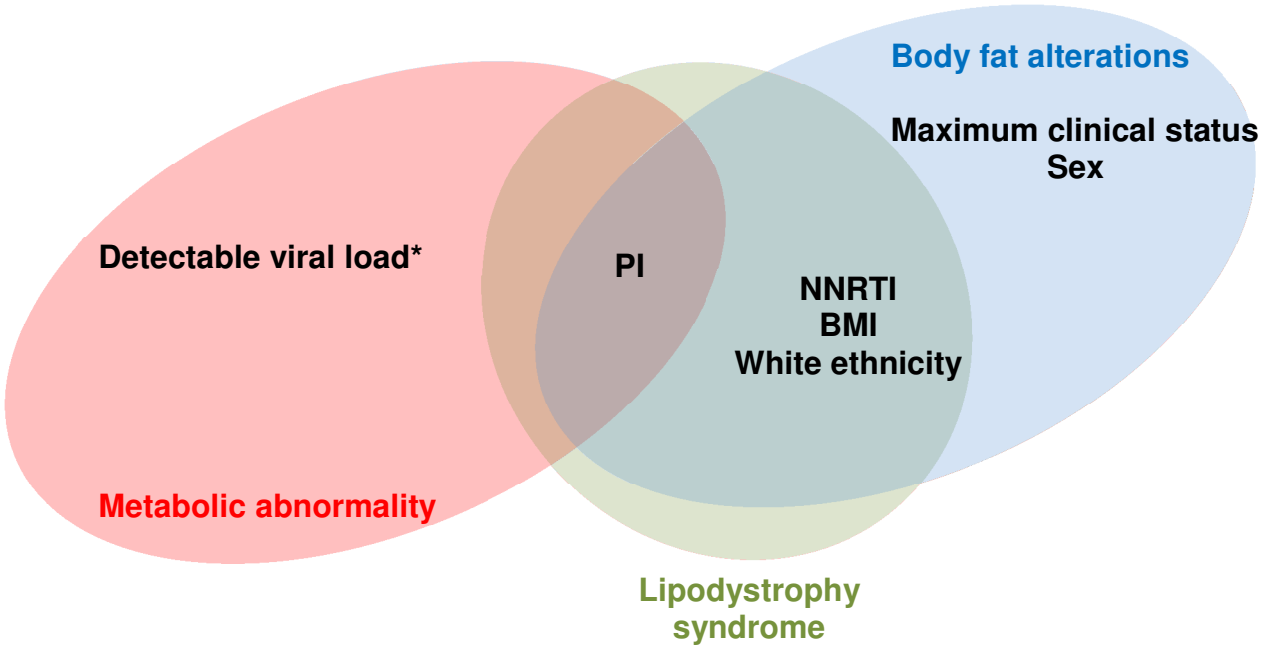
In sensitivity analysis investigating class of ART as explanatory variables where the threshold for stepwise variable selection was raised to 10% significance, independent risk factors were similar. However, maximum clinical status was a significant risk factor for LS in the 10% model (it was not significant in the 5% model): illustrated in Table E-15 in Appendix E.

Comparison of final ART-class models for lipodystrophy syndrome, body fat alterations, and metabolic abnormality

White ethnicity was associated with increased risk in several of the previous multivariable models investigating categories of ART: this risk was statistically significant for any body fat alterations, lipoatrophy, and combined lipoatrophy with lipohypertrophy. Similarly, children currently on any PI were at significantly higher risk of all metabolic abnormality outcomes, and also combined lipohypertrophy with lipoatrophy. While NNRTI use at recruitment was not a significant risk factor for metabolic abnormality outcomes, it was a significant risk factor for all body fat alterations outcomes. Furthermore, BMI was associated with a significant increased risk of any body fat alterations, but was not significantly associated with any other outcome. Figure 5-12 summarizes statistically significant and independent risk factors for lipodystrophy outcomes (metabolic abnormality, body fat alterations or LS), as identified by stepwise logistic regression modelling with classes of ART.

While maximum clinical status was a consistent risk factor for body fat abnormality outcomes, it was not associated with LS in the final multivariable models. Other covariates which had been significant in multivariable models for either body fat alterations outcomes or metabolic abnormality, but not in the final model for LS, were sex (lipohypertrophy) and detectable viral load (hypercholesterolemia).

Figure 5-12: Schematic representation of statistically significant risk factors for lipodystrophy syndrome, body fat alterations, and metabolic abnormality identified by stepwise logistic modelling: models incorporating current ART use by class



Risk factors are significant at the 5% level. Risk factors are associated with increase in risk, except detectable viral load was associated with a decrease in risk . PI and NNRTI use at recruitment, * Associated with a decrease in risk

Final model for specific antiretroviral drugs at recruitment

Table 5-10 illustrates the final multivariable model for LS using specific antiretroviral drugs as explanatory variables. As in the multivariable model investigating class of ART, White ethnicity was associated with a 3-fold increased risk of LS compared to Black ethnicity. BMI was associated with a significant 17% increased risk per 1 unit increase (kg/m^2) while children with a history of symptomatic HIV disease had a significant 2-fold increased risk compared with those who had not experienced HIV symptoms. Use of specific ART drugs were significantly ($p \leq 0.042$) associated with risk of LS: stavudine and efavirenz were associated with a 5-fold and 2-fold increments in risk respectively. However children receiving didanosine at enrolment had a decreased risk of LS which did not reach statistical significance ($p = 0.052$).

Table 5-10: Final multivariable model for lipodystrophy syndrome using specific antiretroviral therapy drugs (*n* = 302)

		n	AOR	95% CI	*p*-value
Age	**Per year**	302	1.08	(0.99, 1.18)	0.102
Maximum duration of ART	**Per year**	302	1.03	(0.96, 1.12)	0.389
Ethnicity	**Black**	73	1		
	White	215	3.32	(1.49, 7.41)	0.003
	Other	14	1.95	(0.46, 8.27)	0.364
Maximum CDC clinical stage	**N/A**	121	1		
	B	110	1.87	(0.99, 3.52)	0.052
	C	71	2.34	(1.18, 4.65)	0.015
BMI	**Per kg/m^2**	302	1.17	(1.06, 1.28)	0.002
Stavudine	**Not current**	266	1		
	Current	36	5.40	(2.15, 13.56)	<0.001
Didanosine	**Not current**	261	1		
	Current	41	0.42	(0.17, 1.01)	0.052
Efavirenz	**Not current**	226	1		
	Current	76	1.93	(1.03, 3.62)	0.042

Model intuitively adjusted for age and duration of ART at recruitment: Adjusted odds ratio (AOR) and 95% confidence interval (95% CI) for lipodystrophy syndrome by participant characteristic. CDC-defined clinical status - N + A: no symptoms/asymptomatic, B: moderate symptoms, C: severe symptoms.

The final adjusted model, developed using stepwise selection of variables including ever-use of specific drugs, was similar to the final model investigating current-use of specific drugs as risk factors for LS (Appendix E: Table E-12). Factors associated with an increase in risk of LS included White ethnicity (AOR: 3.09, 95% CI: 1.77, 5.39), BMI (AOR: 1.11, 95% CI: 1.02, 1.21), and ever-use of stavudine (AOR: 2.24, 95% CI: 1.29, 3.88). However, in contrast to the model presented in Table 5-10, ever-use of didanosine was associated with a significant increased risk of LS (AOR: 1.95, 95% CI: 1.09, 3.49). In addition, ever-use of indinavir was a significant risk factor (AOR: 4.20, 95% CI: 1.08, 16.37), while ever-use of abacavir was associated with a

significant reduced risk (AOR: 0.57, 95% CI: 0.35, 0.93). This protective effect of abacavir may reflect previous regimen switching to this drug after reported reversibility of fat alterations symptoms[238], i.e. confounding by indication. Finally, maximum clinical condition was no longer statistically significant. Further sensitivity analyses were conducted investigating use of specific ART drugs as explanatory variables, but setting the threshold for statistical significance at 5%: in the 5% multivariable model both stavudine and efavirenz were significantly associated with increased risk of LS, while in the 10% model stavudine and ritonavir-booster were significantly associated with increased risk (Appendix E: Table E - 16).

Investigation of puberty as a risk factor for lipodystrophy syndrome

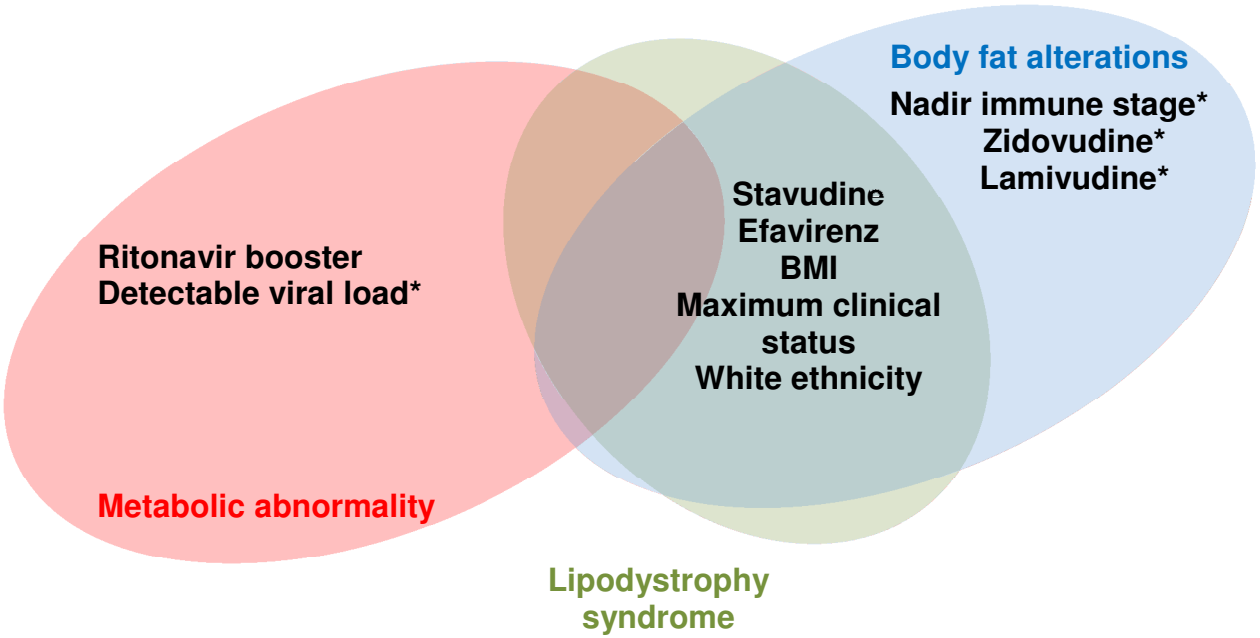
Neither Tanner score nor an interaction between age and sex were statistically significant ($p \geq 0.521$) on addition to the final model for LS using recruitment specific ART as explanatory variables (Table E-13 and Table E-14 in Appendix E).

Comparison of final specific-ART models for lipodystrophy syndrome, body fat alterations, and metabolic abnormality

In the final multivariable models risk factors associated with any fat alterations and lipoatrophy incorporating specific ART drugs, current stavudine was an independent and significant risk factor. Furthermore current efavirenz was a significant risk factor for LS. However stavudine was not a significant risk factor for any metabolic outcome (Figure 5-13). Use of efavirenz at recruitment was associated with a significant increased risk of any fat alterations, and use of didanosine was associated with a non-significant decrease in risk (Chapter 4): these same associations were seen with LS. Although use of ritonavir booster at recruitment was seen as a significant risk factor in multivariable models for all the individual metabolic abnormality outcomes, it was not a significant risk factor for LS or any fat alterations outcomes.

Children and adolescents who had experienced maximum clinical status had increased risk of body fat alterations, lipohypertrophy, and LS in multivariable analysis using specific ART as explanatory variables ($p < 0.05$). However maximum clinical status was not a significant factor in any metabolic abnormality models. White ethnicity was a significant risk factor for any body fat alterations, lipoatrophy and LS in the multivariable models using specific ART drugs, while BMI was an independent risk factor significantly associated with increased risk of body fat alterations, lipohypertrophy, combined lipoatrophy with lipohypertrophy and LS.

Figure 5-13: Schematic representation of statistically significant risk factors for lipodystrophy outcomes, body fat alterations, and metabolic abnormality identified by stepwise logistic modelling: specific antiretroviral drugs as explanatory variables



Risk factors are significant at the 5% level. Risk factors are associated with increase in risk, *risk factors associated with a decrease in risk. Specific drugs refer to drug use at recruitment

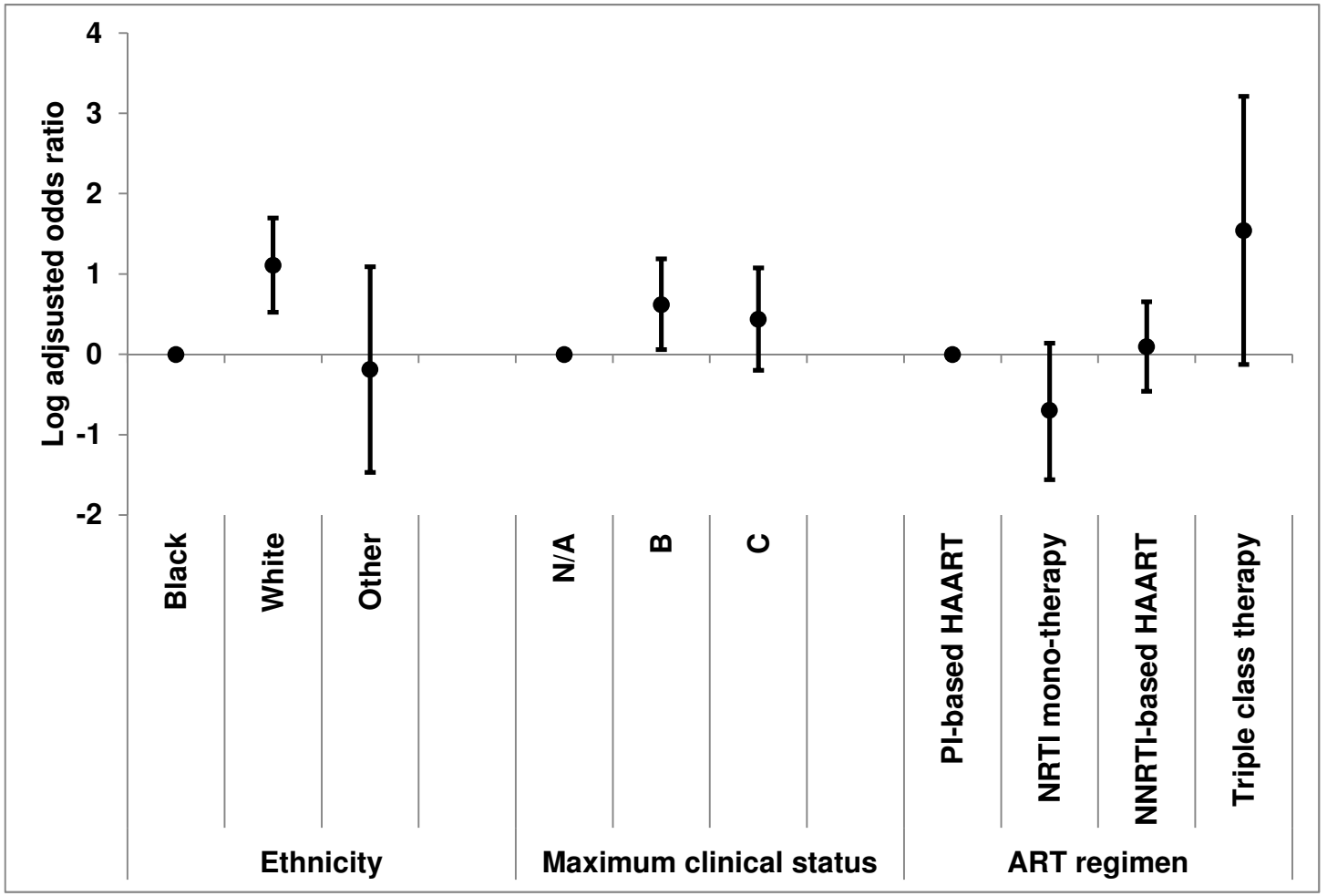
Furthermore, while ritonavir booster and detectable viral load were associated with risk of metabolic abnormality outcomes, and nadir immune status, zidovudine, and lamivudine were associated with body fat alterations outcomes, none of these covariates were significantly associated with LS.

Model for lipodystrophy syndrome including type of ART regimen and comparison with models for metabolic abnormality and for body fat alterations

The multivariable model for LS investigating type of ART, adjusted for age, duration of ART at recruitment, maximum CDC clinical status and ethnicity, is summarized in Figure 5-14. Neither age nor duration of ART was statistically significant in the model ($p \geq 0.477$). Children who had had moderate clinical symptoms at some point during their lifetime had an almost two-fold increased risk of LS (AOR: 1.86, 95% CI: 1.06, 3.28, $p = 0.032$). White ethnicity was associated with a three-fold increased risk of LS (AOR: 3.03, 95% CI: 1.69, 5.45, $p < 0.001$), compared to Black ethnicity.

While NNRTI-based HAART and triple class therapy were associated with increased risk of LS, and NRTI mono-therapy was associated with a decreased risk compared to PI-based HAART, none of these associations reached statistical significance. This is in contrast to fat alterations outcomes where triple class therapy was associated with an independent and significant increased risk of all outcomes, (Chapter 4), and metabolic abnormality outcomes, where NNRTI-based HAART was associated with a reduced risk of all outcomes except hypercholesterolemia, and NRTI-based mono-therapy which was associated with a significant reduced risk of any metabolic abnormality, and fasting hypercholesterolemia.

Figure 5-14: Multivariable model for lipodystrophy syndrome using type of antiretroviral therapy as an explanatory variable



Log adjusted odds ratio displayed for clarity. Model adjusted for age and maximum duration of ART use (not shown), and ethnicity and maximum CDC-defined clinical status. CDC-defined clinical status - N + A: no symptoms/asymptomatic, B: moderate symptoms, C: severe symptoms

5.4 Key points

- Prevalence of metabolic outcomes was higher in older subjects compared to younger subjects, and in pre-pubescent subjects compared to those undergoing or who had completed puberty: these differences were statistically significant

- Undetectable viral load was associated with significantly increased prevalence of any metabolic abnormality, hypercholesterolemia, and both hypercholesterolemia and fasting hypertriglyceridemia occurring together (compared to detectable viral load).

- Subjects on PIs at recruitment had a significantly higher prevalence of all metabolic outcomes compared to those not on these drugs: 90% of subjects on PIs were on boosted ritonavir which was itself associated with significant increased prevalence of all outcomes.

- Prevalence of all metabolic outcomes was highest in subjects on triple class therapy, followed by PI-based HAART, then NNRTI-based HAART and finally NRTI mono-therapy: this was statistically significant for any metabolic abnormality and fasting hypertriglyceridemia.

- Current use of PI at recruitment was an independent, statistically significant risk factor associated with a 2-fold risk of metabolic outcomes, and a 6-fold increased risk of concurrent hypercholesterolemia and fasting hypertriglyceridemia: ritonavir booster was the only specific drug significantly associated with risk of each outcome (<8-fold increase).

- Current NNRTI-based HAART was associated with a significant decreased risk of any metabolic abnormality, fasting hypertriglyceridemia, and concurrent hypercholesterolemia and fasting hypertriglyceridemia, compared to PI-based HAART.

- A significant and independent 60% decreased risk of hypercholesterolemia was seen with detectable viral load. Age was associated with a reduction in risk of metabolic abnormality outcomes. Statistically significant risk factors for metabolic outcomes are summarized in Table 5-11.

Table 5-11: Summary of risk factors for metabolic outcomes across all final multivariable models

	Metabolic abnormality	Fasting hypertriglyceridemia	Hypercholesterolemia	Both fasting hypertriglyceridemia and hypercholesterolemia
Age*	↓	↓	↓	↓
Maximum duration of ART*				↑
Detectable viral load			↓	
PI	↑	↑	↑	↑
Ritonavir booster	↑	↑	↑	↑
NRTI mono-therapy**	↓	↓		
NNRTI-based HAART**	↓	↓		↓

Only factors that were statistically significant in at least one individual model are shown. Arrows indicate direction of association. *Unit: ascending year, **comparison group: PI-based HAART.

- The prevalence of LS was significantly higher in: subjects of White ethnicity compared to those of Black ethnicity; those who were immunosuppressed at recruitment compared to those who were not; subjects who had a history of CDC-defined symptomatic disease compared to subjects who did not; and subjects with non-detectable viral load at recruitment compared to those who have detectable viral load.

- Prevalence of LS was significantly higher in subject on PI, compared to those not on these drugs at recruitment. The percentage of the study population with LS was highest in subjects on triple class therapy followed by those on HAART, and lowest in subjects on NRTI mono-therapy. However, numbers of subjects on triple class therapy at recruitment was very small (n = 13)

- In multivariable models, White ethnicity, BMI, and maximum CDC-defined clinical condition were associated with increased risk of LS (<4 fold, 10-17% per kg/m^2, 2-fold respectively).

- Both use of PI and NNRTI at recruitment were significantly associated with a 3-fold increased independent risk of LS, with stavudine associated with a 5-fold increased risk, and efavirenz associated with a 2-fold increased risk. However, current didanosine was associated with a statistically significant 58% decreased risk

6. Factors associated with the incidence of lipodystrophy syndrome

6.1 Objectives

This chapter describes the cohort at the end of the follow-up period, investigating the incidence of LS in subjects without symptoms at recruitment, and identifying factors associated with its emergence.

Specific objectives include:-

- Description of the longitudinal dataset including changing clinical and treatment characteristics.
- Estimation of the prevalence of LS outcomes at the end of follow-up.
- Estimation of incidence of fat alterations, metabolic abnormality, and LS over follow-up.
- Investigation of recruitment factors and changing clinical factors over follow-up associated with the incidence of body fat alterations, metabolic abnormality, and LS.
- Examination of regression or progression of symptoms of LS in subjects who had the syndrome at recruitment.

6.2 Specific methods

6.2.1 Estimation of prevalence at the end of follow-up

The prevalence of LS, and its component phenotypes (Table 6-1), at the end of the follow-up period was estimated using methods as described in previously.

Table 6-1: Longitudinal analyses: outcome measures

Measure	Prevalence at end of follow-up	Incidence rate during follow-up	Risk factors
Lipodystrophy syndrome	**X**	**X**	**X**
Components of lipodystrophy syndrome			
Any body fat alterations	**X**	**X**	**X**
Any lipoatrophy	**X**	**X**	
Any lipohypertrophy	**X**	**X**	
Any metabolic abnormality	**X**	**X**	**X**
Any hypercholesterolemia	**X**	**X**	
Any fasting hypertriglyceridemia	**X**	**X**	

6.2.2 Definition of incident lipodystrophy syndrome

The outcomes that were investigated for the incidence of LS symptoms are illustrated in Table 6-1. Data collection occurred at up to four points. However, not all subjects had data collected at four time points, e.g. Individual C in Figure 6-1. Indeed, no subject who was resident in Italy had more than three data collection points.

A subject was newly diagnosed with LS at a follow-up study visit where he/she was known not to have LS at the previous study visit[h]. For example, as illustrated in Figure 6-1, subject A was assessed as not having LS symptoms at recruitment, and the second visit, but did have LS symptoms at third visit and the fourth visit: this subject was classed as having incident LS at the third visit. Complete reversal of LS was defined as a subject going from a state of metabolic abnormality to acceptable cholesterol/fasting triglyceride/glucose tolerance and/or going from having body fat alterations to no alterations (e.g. Subject B in Figure 6-1 goes from having prevalent LS at recruitment to complete reversal at the second study visit).

Assumptions were made about missing data at a study visit. Where LS data were missing at a follow-up visit, but were available from the previous study visit and at the subsequent follow-up,

[h] Since all time-to-event analyses aimed to investigate the emergence of LS symptoms: all subjects with prevalent symptoms were excluded and a structure of right censoring was assumed. Examination of when symptoms may have appeared in prevalent cases was not an objective of in this investigation, and was not required for modelling incidence in the susceptible population, Thus, interval censoring was not used to assume the point at which symptoms occurred between study visits.

the LS status of the subject was assumed to be the same as at the previous study visit (e.g. Subject E in Figure 6-1). For example, subject B in Figure 6-1, was assessed to have LS symptoms at recruitment, no symptoms at the second study visit, data was missing at the third visit, and no symptoms at the fourth visit: since the LS status was known at the second study visit and the fourth visit, subject B was assumed to have no LS symptoms between these visits. This assumption was only applicable to 5 subjects: 1 subject was assumed to have LS at a hospital visit where data was missing, and 4 were assumed to have no LS symptoms at hospital visits with missing data. Right censoring was used in the development of assumptions regarding LS status over follow-up: it was assumed that symptoms (or lack of symptoms) were consistent for an individual unless otherwise known. Thus for Subject C, (s)he was known not to have LS at his/her last study visit (visit 3), and it was therefore assumed that (s)he did not develop LS by the end of follow-up as no contradictory evidence had been collected. Because of these assumptions, the size of the population at the end of follow-up can be comparable to the size at the beginning, regardless of the number of follow-up visits. Section 2 of Appendix F illustrates this approach further and includes sensitivity analyses conducted to investigate these assumptions.

Date of onset of symptoms (incidence), and date of complete reversal of symptoms, was estimated as the date of completion of the data collection form which coincided with the study visit when the diagnosis was made.

6.2.3 Estimation of incidence

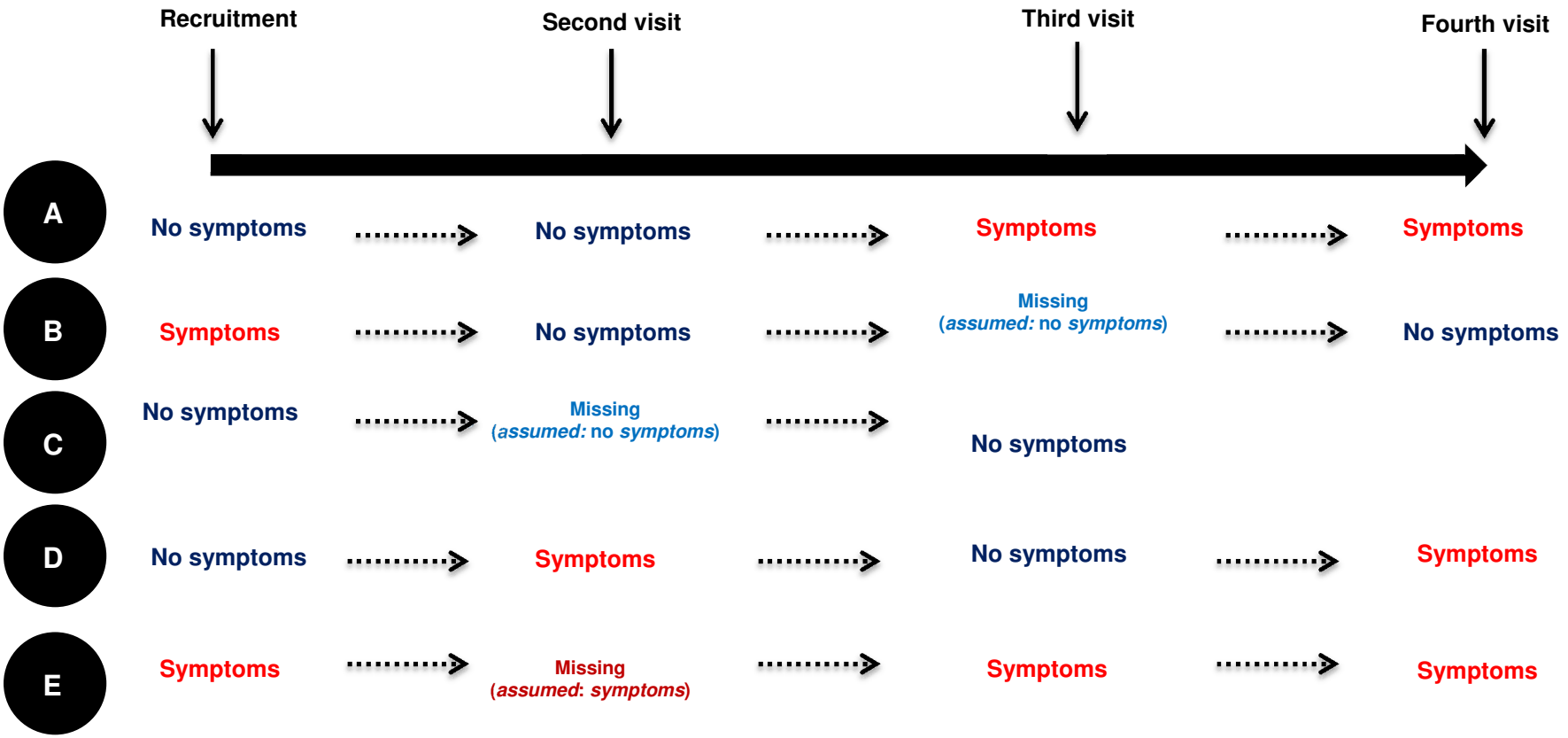
Incidence was estimated from the study population who were free of any symptoms of LS outcomes at recruitment: subjects who had, or were assumed to have prevalent LS symptoms at recruitment were excluded from both estimation of incidence, and Cox regression models. For estimation of incidence of each LS outcome, the number of excluded subjects was as follows: body fat alterations; n = 172, lipohypertrophy; n = 122 = 114, lipoatrophy; n = 122 = 113, metabolic abnormality; n =87, fasting hypertriglyceridemia; n = 122 = 38, hypercholesterolemia; n = 122 = 54, and LS; n = 228. Examples of prevalent cases include subjects B and E in Figure 6-1. This ensured that the denominator person-years in estimation of incidence was calculated correctly, and that the Cox models were comparing incident cases to non-cases as opposed to incident cases to a mixture of cases and non-cases.

Subjects who had more than one change in LS status during follow-up (e.g. subject D in Figure 6-1) were not included in the estimation of incidence. Such cases were also excluded from Cox regression modelling (body fat alterations; n = 19, metabolic abnormality; n = 20, and LS; n = 17). Including cases with this added complexity would make inferences from the models more difficult.

However, it is important to note that incident cases of either body fat alterations or metabolic abnormality can occur in prevalent cases of LS. For example, a subject can have body fat alterations, but no metabolic abnormality at recruitment and thus be a prevalent case of LS, but would be considered “at-risk” for the emergence of metabolic abnormality. This same subject would contribute to the denominator for estimation of incidence of metabolic abnormality, but not to the denominator of estimation of body fat alterations incidence. If this same subject was to later develop fasting hypertriglyceridemia, s(he) would be an incident case of metabolic abnormality, i.e. also contribute to the numerator for incident metabolic abnormality. Similarly a subject with fasting hypertriglyceridemia and no body fat alterations at recruitment would be categorized as a prevalent case of LS (at recruitment) but susceptible for incident body fat alterations (during follow-up): if (s)he developed lipoatrophy over the follow-up period, the subject would be included in estimation of the rate of incidence of body fat alterations.

Incidence of each component of LS (Table 6-1) was estimated as the number of subjects with incident symptoms per 100 years of follow-up time (person-time). Total person-time was estimated as the sum of the interval between date of recruitment and date of the last follow-up for each subject. Person-time at risk for incidence of LS was calculated as the difference between the date of recruitment and the date diagnosis in incident cases. Thus the total person-time at risk for incidence of LS was the sum of each individual person-time at risk. In the same way, total person-time at risk for reversal of LS symptoms was the sum of the individual differences between date of recruitment and date of report of complete reversal of symptoms (or the date of last follow-up if the patient did not experience full reversal of symptoms).

Figure 6-1: Follow-up of subjects with respect to lipodystrophy syndrome status



Subject A is a case of incident lipodystrophy syndrome, subject B has complete reversal of lipodystrophy syndrome, subject D has both incident lipodystrophy syndrome and regression of symptoms to pre-LS state, and subject E has consistent symptoms. Assumptions are made about the symptoms of subjects B, C and E over the follow-up period. Subjects D and E are excluded from Cox regression models.

6.2.4 Time to event (incident lipodystrophy syndrome) analysis

Baseline factors that were significantly associated with risk of fat alterations, metabolic abnormality and LS in logistic regression models (Chapters 4 and 5) are summarized in Table 6-2: two sets of multivariable analyses were conducted: (i) models investigating variables at recruitment, and (ii) models investigating time-updated variables.

Table 6-2: Explanatory variables in Cox regression models

Variable	Models investigating factors at recruitment	Models investigating time updated variables
Gender	X	X
Ethnicity	X	X
Detectable viral load at recruitment	X	
Maximum CDC-defined clinical status	X	
Body mass index at recruitment	X	X
Protease inhibitor use at recruitment	X	X
Non-nucleotide reverse transcriptase inhibitors at recruitment	X	X
Detectable viral load during follow-up		X
CDC-defined HIV symptoms during follow-up		X
CDC-defined HIV immunosuppression during follow-up		X

The probability of incidence of LS symptoms during follow-up associated with each of the factors in Table 6-2 was investigated by using Kaplan-Meir survival estimates.

The association between recruitment factors and incidence was assessed using Cox proportional hazard models. Both univariable and multivariable models were explored. Each multivariable model initially contained all factors included in Table 6-2, and were additionally adjusted for age and duration of ART use at recruitment. Thresholds used to define underweight, normal weight, and overweight and obesity were WHO BMI cut offs for adults[390-392] (Table 6-3). Each multivariable model contained a random effect for clinical site. A backwards covariate selection procedure was conducted to ascertain covariates in the final model as outlined in Chapter 2. All final models retained age, duration of ART use at recruitment and the random effect for clinical site regardless of their statistical significance in the model.

Additional multivariable Cox regression models were fitted to investigate ART regimen (classified as PI-based HAART, NRTI mono-therapy, NNRTI-based HAART, and triple class therapy): these models were adjusted for both age and duration of ART use at recruitment, and also contained a random effect term for clinical site at recruitment.

Table 6-3: International classification of adult underweight, overweight and obesity according to body mass index

	BMI range (kg/m^2)
Severely underweight	<16.00
Underweight	16.0 – 18.5
Healthy weight	18.5 – 25.0
Overweight	25.0 – 30.0
Obese	>30.0

Thresholds for adults defined by the World Health Organization (WHO)[390-392]

Further univariable and multivariable models were constructed to investigate the association with time-updated covariates over follow-up and the incidence of LS outcomes. Table 6-2 illustrates the covariates examined in these models.

Sensitivity analyses were conducted by repeating the stepwise covariate selection process but relaxing the criteria for inclusion of variables to 10% statistical significance. No difference was seen between the 5% and the 10% multivariable models.

6.2.5 Exploration of progression or regression of lipodystrophy syndrome symptoms

Subjects who were diagnosed as having LS at recruitment, but who had subsequently had a change in severity of symptoms over the follow-up period, were identified. Eight (8) specific outcomes, 4 related to body fat alterations, and 4 to metabolic abnormality were investigated

Regression of body fat alterations was defined as a subjects having at least one of the following:

- having been diagnosed as having severe fat alterations at a given study visit and then diagnosed as having moderate, mild or no symptoms at the subsequent visit;
- having moderate fat alterations at a given study visit and then diagnosed as having mild or no symptoms at the subsequent hospital visit
- having mild symptoms at a given study visit and subsequently having no symptoms.

Similarly, progression of symptoms of body fat alterations was defined as at least one of:

- no symptoms at a given study visit and mild/moderate/severe symptoms at the subsequent hospital visit;
- mild symptoms at a given study visit followed by either moderate/severe symptoms at the subsequent study visit
- moderate symptoms at a given study visit followed by severe fat alterations at the subsequent study.

Regression of metabolic abnormality was defined as the subject:

- having been diagnosed as having LS at a given study visit, and then seeing a 20% decrease in serum cholesterol and/or 20% decrease in serum fasting triglyceride at the subsequent study visit.

Progression of metabolic abnormality was defined as the subject:

- having been diagnosed as having LS at a given study visit, and then seeing a 20% increase in cholesterol and/or 20% increase in serum fasting triglyceride at the subsequent study visit.

Thus, examination of progression/regression of LS symptoms was not only restricted to those subjects who had LS at recruitment, but could also include both examples where symptoms may have disappeared completely (in investigation of regression of symptoms) or examples of incident cases following previous complete reversal of symptoms (in investigation of both progression and regression of symptoms).

6.3 Results

6.3.1 Follow-up of the cohort

Of the 426 children and adolescents enrolled into the cohort, prospective data were collected up to close of follow-up (at the end of December 2011) from 388 (83.3%) subjects, i.e. 38 subjects (21 of whom had LS symptoms) attended only 1 study visit (Table 6-4). Over 3% of the cohort had data collected at two study visits, and over half (55.2%, *n* = 235) had attended three study visits. Furthermore, 88 subjects (20.1%) had attended 4 study visits (all were resident in Belgium and Poland); no Italian-resident subjects had >3 visits during follow-up. Median follow-up for the cohort was 4.19 years (IQR: 3.00, 4.59), and was 3.25, 3.00 and 4.47 years (Table 6-4) for Belgian-, Poland- and Italy-resident subjects respectively. The median time-lag between study visits for Poland/Belgian residents was between 0.50 and 0.65 years, while the median difference in Italian residents was over 1 year (Appendix F: Table F-3). Both the differences in total follow-up, and the differences in study visit intervals, were significant between countries ($p < 0.001$).

Table 6-4: Number of hospital visits by country of residence

	Total number of subjects at recruitment	1 visit		2 visits		3 visits		4 visits		Median total follow up* (IQR**)
		%	*n*	%	*n*	%	*n*	%	*n*	
Belgium	84	*1.19*	1	*3.57*	3	*21.43*	18	*73.81*	62	3.25 (1.69, 3.81)
Italy	275	*22.54*	33	*2.91*	8	*74.55*	28	*0*	0	4.47 (4.05, 4.75)
Poland	67	*11.94*	4	*2.99*	2	*17.91*	12	*67.16*	45	3.00 (2.63, 3.07)
TOTAL	426	*16.67*	38	*3.05*	13	*55.16*	268	*25.12*	107	4.19 (3.00, 4.59)

*** Median interval between first and last clinical visits in years. **Inter quartile range. Mann-Whitney-U test for medians between countries: $p < 0.001$**

Antiretroviral therapy use through follow-up

Of the 29 subjects who were ART-naïve at recruitment (Chapter 3), all commenced ART at some point during follow-up, i.e. no subject was ART-naïve at the end of follow-up. Of these subjects, 1 commenced with NNRTI-based HAART, and 2 commenced on PI-based HAART. Interestingly, 26 commenced on NRTI mono-therapy (lamivudine): 10 subsequently started NNRTI-based HAART by the end of follow-up, while no subsequent data was available for the remaining 16 (all Italian-resident). At the end of follow-up, 5 of the 29 subjects who were ART-naïve at recruitment had symptomatic HIV disease, 22 had detectable viral load, but no subject

had immunosuppression. Furthermore, data on ART was collected during following up for all 33 subjects whose ART status was unknown at recruitment (Appendix F: Figure F-5)[i].

Overall, 27.4% of subjects (*n*/*N* = 106/387) experienced at least one change in the type of ART regimen (i.e. change between PI/NNRTI-based HAART/triple class therapy/NRTI mono-therapy), and all subjects who were being treated at recruitment were continuing with ART at the end of follow-up.

Changing HIV-disease status during follow-up

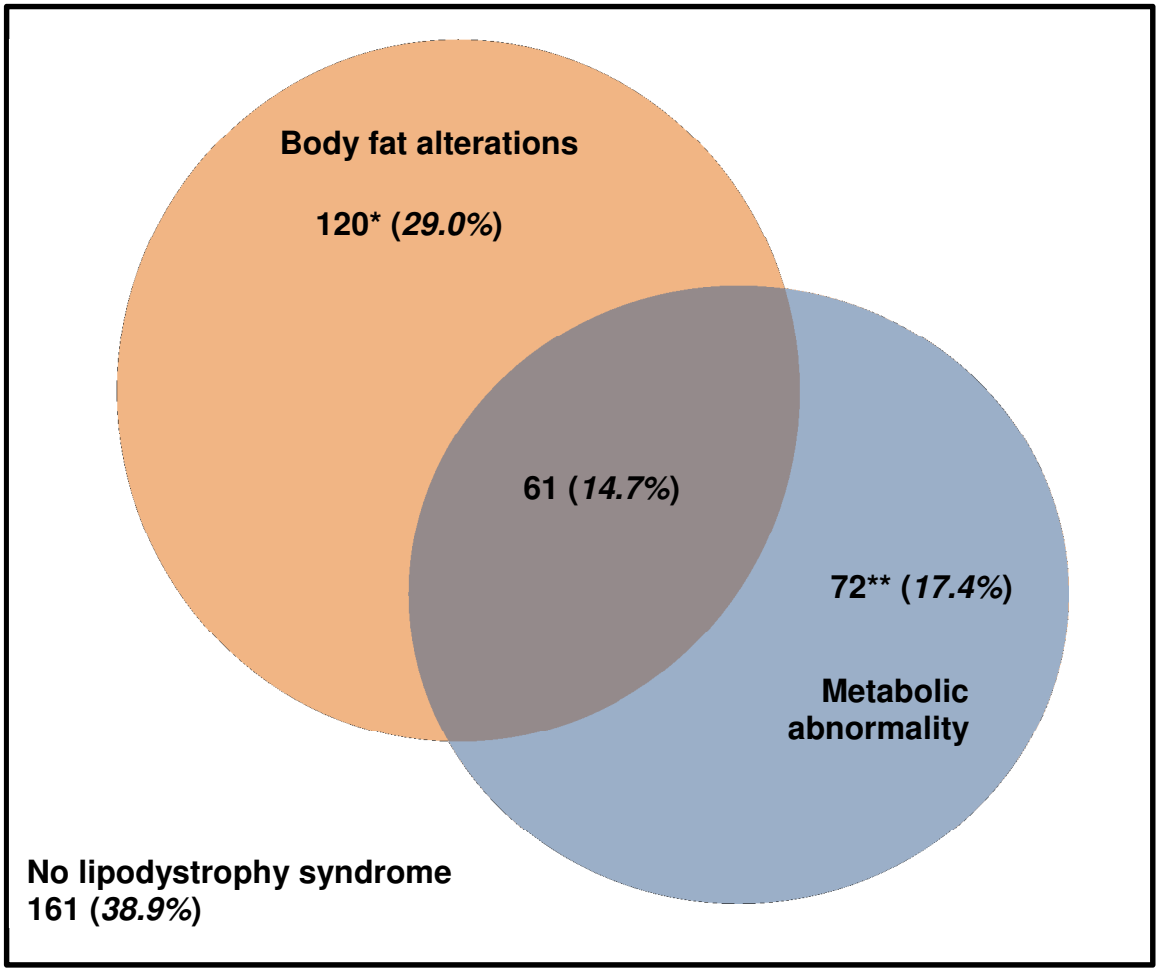
At the end of follow-up, the prevalence of detectable viral load was 53.1% (*n*/*N* = 221/416), of symptomatic HIV disease was 28.1% (*n*/*N* = 115/410) and of moderate or severe immunosuppression was 5.5% (*n*/*N* = 23/416), as illustrated in Figures F-2 to F-4 in Appendix F. Thus, while there were increases in prevalence of detectable viral load and clinical symptoms since recruitment, this was accompanied by a decrease in the prevalence of immunosuppression. At the end of follow-up, the prevalence of subjects with at least one of detectable viral load, symptomatic disease, or immunosuppression was 70.0% (*n*/*N* = 287/410), with the prevalence of subjects with either symptomatic disease or severe immunosuppression being 30.0% (*n*/*N* = 123/410). The prevalence of subjects with all three was 0.5% (*n*/*N* = 2/410).

6.3.2 Prevalence of lipodystrophy syndrome at the end of follow-up

At the end of the follow-up period, the prevalence of LS was 61.1% (95% CI: 56.4, 65.8, *n*/*N* = 253/414), with the prevalence of fat alterations alone being 29.0% (95% CI: 24.6, 33.4, *n*/*N* = 120/414), of metabolic abnormality alone being 17.3% (95% CI: 13.7, 21.0, *n*/*N* = 72/414), and of both body fat alterations and metabolic abnormality was 14.7% (95% CI: 11.3, 118.1, *n*/*N* =61/414) as illustrated in Figure 6-2.

[i] Data was only collected on change in ART use over follow-up: thus these 33 subjects were known to be on ART during follow-up, but no information on past ART use, if known, was collected.

Figure 6-2: Prevalence of lipodystrophy syndrome at the end of follow-up: metabolic abnormality and body fat alterations (*n* = 414)



***Includes 18 subjects with body fat alterations at the end of follow-up, but whose metabolic abnormality status was unknown. **Includes 1 subject with metabolic abnormality at the end of follow-up, but whose body fat alteration status was unknown.**

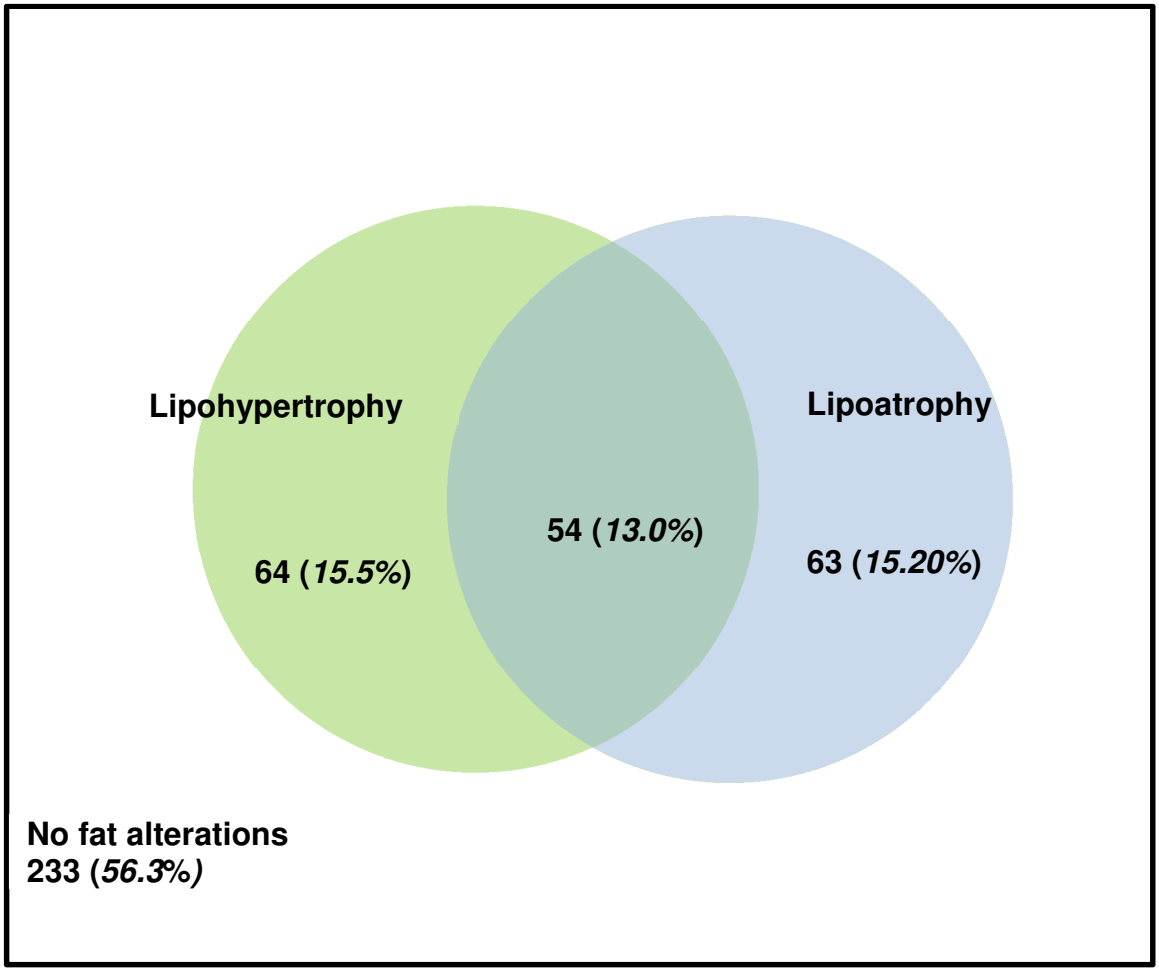
There were increases in prevalence of LS, body fat alterations and metabolic abnormality between recruitment and the final follow-up, as illustrated in Table 6-5. However, as at recruitment, nearly half (47.4%) of final-round LS cases were defined by body fat abnormality alone, just over one-quarter (28.5%) by metabolic abnormality alone, and almost one-quarter (24.1%) by the combined metabolic abnormality and fat alterations occurring together.

Of the 181 children and adolescents with fat alterations at the end of follow-up, 34.8% (*n* = 63) had lipoatrophy alone, 35.4% (*n* = 64) had lipohypertrophy alone and 29.8 (*n* = 54) had both (Figure 6-3): this distribution of body fat alteration symptoms was similar to that seen at recruitment.

Table 6-5: Comparison of prevalence of lipodystrophy syndrome outcomes at recruitment and at end of follow-up

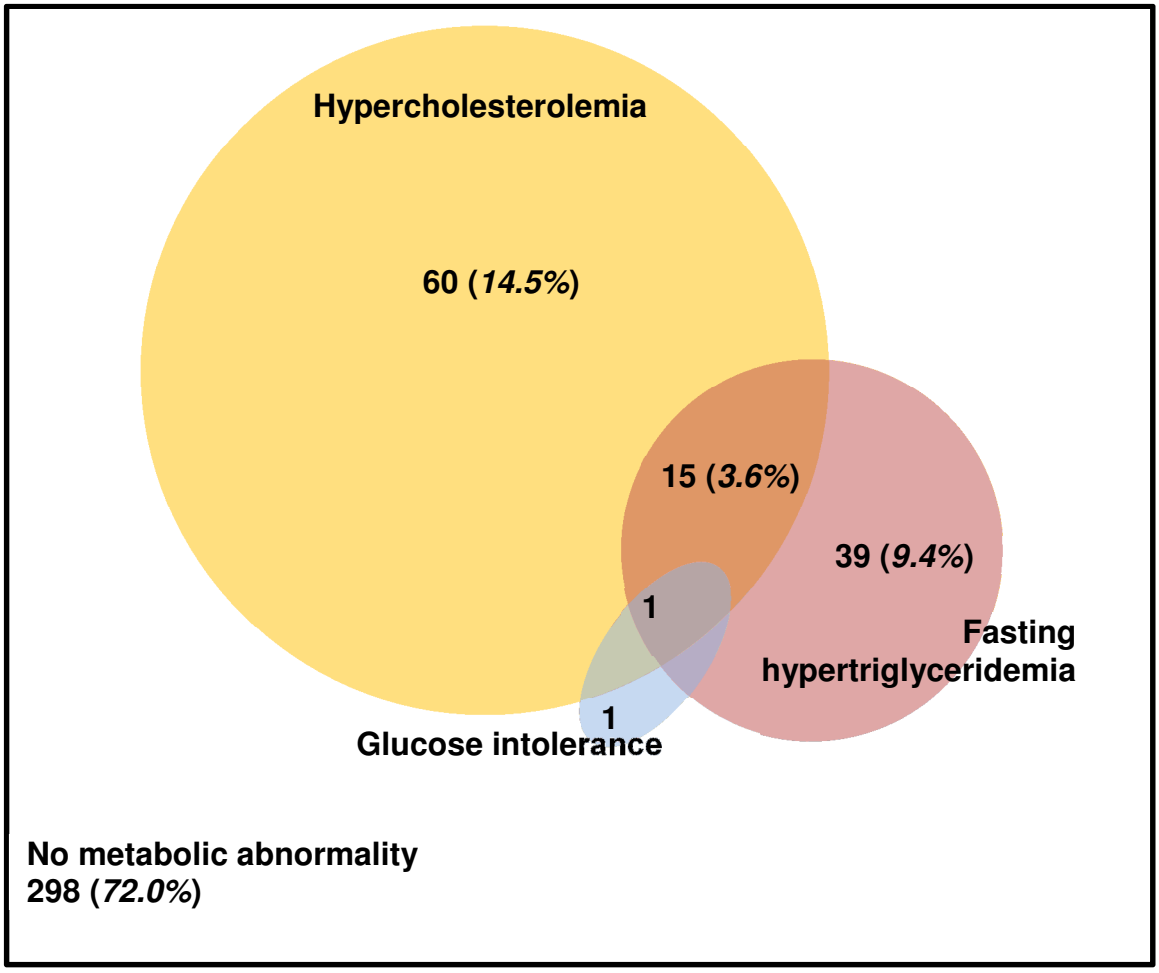
	Recruitment		End of follow-up	
	n*/*N	**Prevalence (95% CI)**	***n*/*N***	**Prevalence (95% CI)**
Lipodystrophy syndrome	*235/416*	56.5 (51.7, 61.3)	*253/414*	61.1 (56.4, 5.8)
Any fat alterations	*176/422*	41.7 (37.0, 46.4)	*181/413*	43.8 (39.0, 48.8)
Any lipohypertrophy	*115/422*	27.3 (23.0, 31.5)	*118/414*	28.5 (24.2, 33.1)
Any lipoatrophy	*117/422*	27.7 (23.5, 32.0)	*117/413*	28.3 (24.0, 32.9)
Concurrent lipohypertrophy and lipoatrophy	*56/422*	13.3 (10.0, 16.5)	*54/413*	13.1 (10.0, 16.7)
Any metabolic abnormality	*108/416*	26.0 (21.6, 30.0)	*116/414*	28.0 (23.8, 32.7)
Any fasting hypertriglyceridemia	*71/419*	16.9 (13.4, 20.5)	*55/414*	13.3 (10.3, 17.0)
Any hypercholesterolemia	*56/419*	13.4 (10.1, 16.6)	*76/414*	18.4 (14.8, 22.5)
Concurrent hypercholesterolemia and fasting hypertriglyceridemia	*23/419*	5.5 (3.1, 7.4)	*16/414*	3.9 (2.3, 6.3)
Glucose intolerance	*7/419*	1.7 (0.4, 3.0)	*2/414*	0.5 (0.0, 1.9)

Figure 6-3: Prevalence of body fat alterations outcomes at the end of follow-up: lipoatrophy and lipohypertrophy (*n* = 414)



The proportions of subjects with each manifestation of metabolic abnormality varied between recruitment and the end of follow-up (Figure 6-4). While only 29.6% of subjects with metabolic abnormality had hypercholesterolemia alone at recruitment, this had increased to 51.7% at the end of follow-up. In contrast, the proportions of subjects with fasting hypertriglyceridemia alone, hypercholesterolemia with fasting hypertriglyceridemia, and glucose intolerance alone had decreased from 43.5% to 33.6% (*n* = 39), 20.4% to 12.9% (*n* = 15), and 3.7% to 1.0% (*n* = 1) respectively. While there had been 3 cases of mixed phenotype including glucose intolerance at recruitment, by the end of follow-up mixed phenotype this was reduced to 1.

Figure 6-4: Prevalence of metabolic abnormality outcomes at the end of follow-up: hypercholesterolemia, fasting hypertriglyceridemia and glucose intolerance (*n* = 414)



6.3.3 Incidence of lipodystrophy syndrome outcomes over follow-up

Total person-years of follow-up was 1318.7, with almost 70% (923.0 person-years) contributed by the 276 Italian subjects and over 30% (395.7 person-years) contributed by 150 Polish/Belgium subjects.

Rates of incidence of new cases of body fat alterations are summarized in Table 6-6 and Figure 6-5. The rate of incidence of new cases of LS (i.e. body fat alterations and/or metabolic abnormality) was 17.8 cases per 100 person years (95% CI: 14.1, 21.5), i.e. 74 subjects developed LS over 416.2 person-years at risk. New cases of body fat alterations occurred at a rate of 8.0 cases per 100 person years (95% CI: 6.0, 10.7), i.e. 45 cases over 561.7 person-years at risk. Over the follow-up period, 29 new cases of metabolic abnormality occurred during 712.9 person-years at risk: this was equivalent to a rate of 4.1 cases per 100 person-years (95% CI: 2.8, 5.9).

The rate of incidence of new cases of lipoatrophy was 5.9 per 100 person-years (95% CI: 4.3, 8.0), i.e. 42 cases over 710.8 person years at risk: lipoatrophy emerged most commonly in the buttocks with a rate of 5.3 cases per 100 person-years (95% CI: 3.9, 7.2: 43 cases over 806.5 person-years at risk). Rates of incidence of lipoatrophy and of lipohypertrophy were similar: the rate of lipohypertrophy was 5.2 cases per 100 person-years of follow-up (95% CI: 3.7, 7.2): equivalent to 36 new cases over 687.8 person-years at risk. Although rates of lipohypertrophy incidence in the breast and trunk were similar, the magnitude of the latter was slightly higher (3.9 per 100 person-years at risk, 95% CI: 2.7, 5.7: 28 cases with 714.0 person-years at risk.

Over 830.7 person-years at risk, 16 subjects developed fasting hypertriglyceridemia, i.e. a rate of incidence of 1.9 cases per 100 person-years (95% CI: 1.2, 3.2), as illustrated in Figure 6-6. The number of incident new cases of hypercholesterolemia was 37: over 798.9 person-years of risk, this was equivalent to a rate of 4.6 per 100 person-years (95% CI: 3.3, 6.4).

Table 6-6: Rates of (i) incidence of new cases lipodystrophy outcomes, and (ii) complete reversal of symptoms in prevalent cases

	Rate of incidence of new cases			Rate of reversal of prevalent cases		
	Number of events	Person years at risk	Rate (95% CI)	Number of events	Person years at risk	Rate (95% CI)
Body fat alterations	45	561.72	8.01 (5.96, 10.65)	38	387.17	9.81 (7.13, 13.34)
Lipoatrophy	42	710.80	5.91 (4.34, 7.97)	37	315.12	11.74 (8.51, 15.95)
Lipohypertrophy	36	687.77	5.2 (3.7, 7.2)	34	257.53	13.2 (9.4, 18.1)
Metabolic abnormality	29	712.93	4.07 (2.79, 5.86)	42	488.98	8.59 (6.33, 11.52)
Fasting hypertriglyceridemia	16	830.70	1.93 (1.15, 3.18)	29	459.93	6.31 (4.33, 9.03)
Hypercholesterolemia	37	798.94	4.63 (3.32, 6.39)	27	178.87	15.09 (10.34, 21.36)

Incidence per 100 years of person-time follow-up

6.3.4 Complete reversal of lipodystrophy syndrome

Among subjects with prevalent LS, 80 experienced complete reversal of symptoms equivalent to pre-LS levels: this corresponded with a rate of reversal of 12.7 per 100 person-years (95% 10.2, 15.6: 629.7 person-years at risk). The rate of complete reversal of body fat alterations (9.8 per 100 person-years, 95% CI: 7.1, 13.3; 38 cases over 387.2 person-years) was greater than the rate of reversal of metabolic abnormality (8.6 per 100 person-years, 95% CI: 6.3, 11.5; 42 cases over 489.0 person-years at risk). Rates of complete reversal of LS symptoms in prevalent cases are summarized in Table 6-6, Figure 6-5 and Figure 6-6.

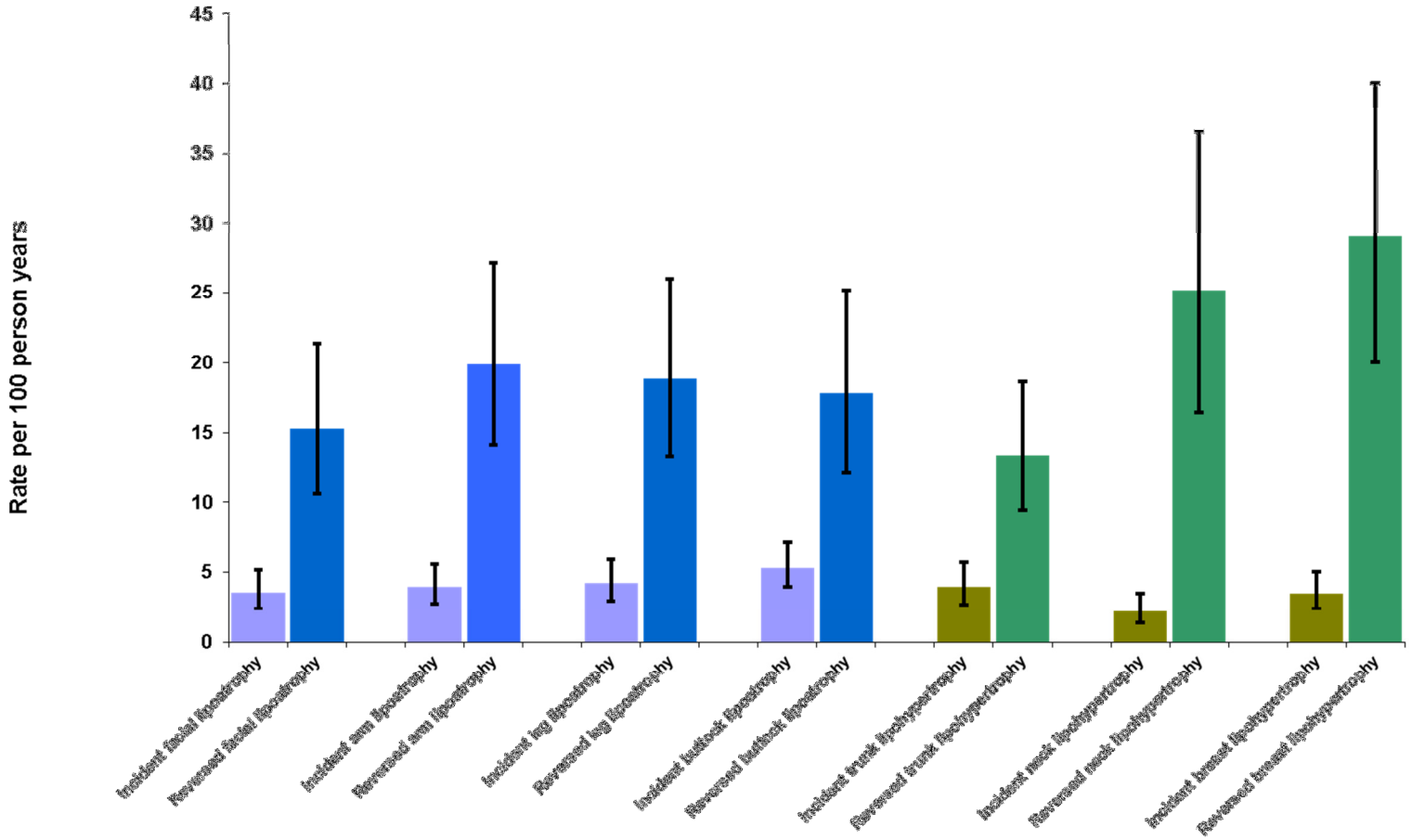
The rate of complete reversal of lipoatrophy was 11.7 cases per 100 person-years (95 % CI: 8.5, 16.0; 37 cases over 315.1 person-years of risk), with the greatest rate seen in the arm (19.9 cases per 100 person-years, 95% CI: 14.1, 27.2; 31 cases over 156.0 person-years at risk). However, the rate of complete reversal of lipohypertrophy was higher: 13.2 per 100 person-years (95% CI: 9.4, 18.1; 34 cases over 257.5 person-years). The rate of complete reversal of fasting hypertriglyceridemia was 6.3 cases per 100 person-years (95% CI: 4.3, 9.0), i.e. 29 cases over 459.9 person-years at risk. Of the prevalent cases of hypercholesterolemia, 27 experienced normalization of cholesterol levels: the rate of complete reversal of symptoms was 15.1 per 100 person-years (95% CI: 10.3, 21.4; 178.9 person-years at risk).

6.3.5 Subjects with more than one change in lipodystrophy syndrome status over follow-up

Less than one tenth of the cohort, (n = 44) had more than one change in their LS status over the follow-up period, (e.g. subject D in Figure 6-1). Of these 44 subjects, 19 (43.2%) were defined by having more than one change in fat alterations status over the follow-up period, while 45.5% (n = 20) were defined by having more than one change in metabolic abnormality status (Figure 6-7). The remaining 11.4% (n = 5) had more than one change in both fat alterations and metabolic abnormality status.

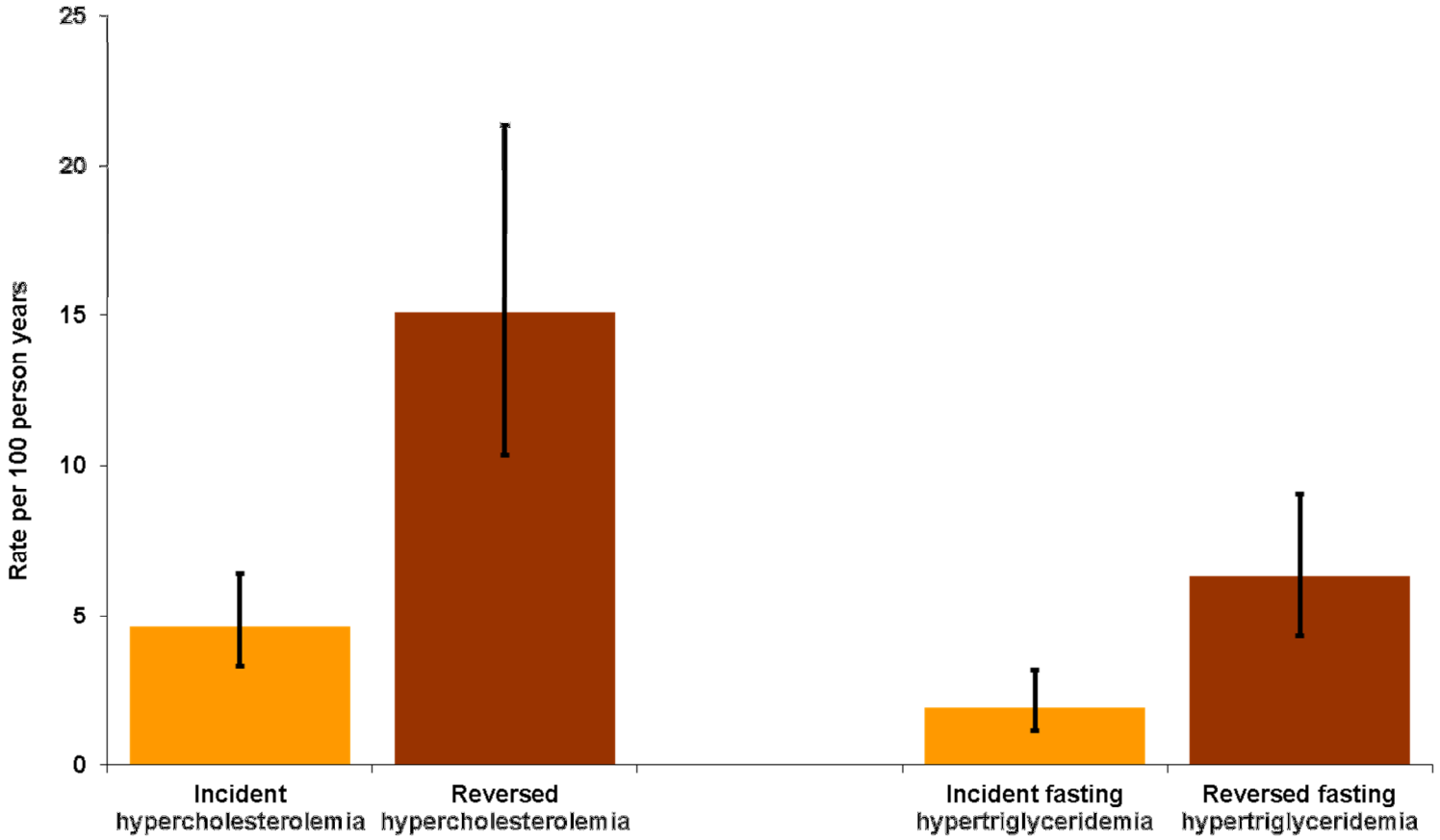
The median age at recruitment of these 44 subjects was 13.5 years (IQR: 11.1, 13.5), and duration of ART at recruitment was 4.9 years (IQR: 1.9, 4.9): there was no statistically significant difference in either recruitment age, nor recruitment duration of ART between this group and subjects who were consistently free of LS symptoms during follow-up. Similarly, no significant association ($p > 0.05$) was seen between subjects with >1 change in LS symptoms and those with no symptoms throughout follow-up with respect to maximum and recruitment CDC-defined clinical status, or recruitment degree of immunosuppression. However, a significant association was seen between nadir degree of immunosuppression and having >1 change in LS status ($p = 0.039$).

Figure 6-5: Rates of incidence or complete reversal of specific body fat alterations



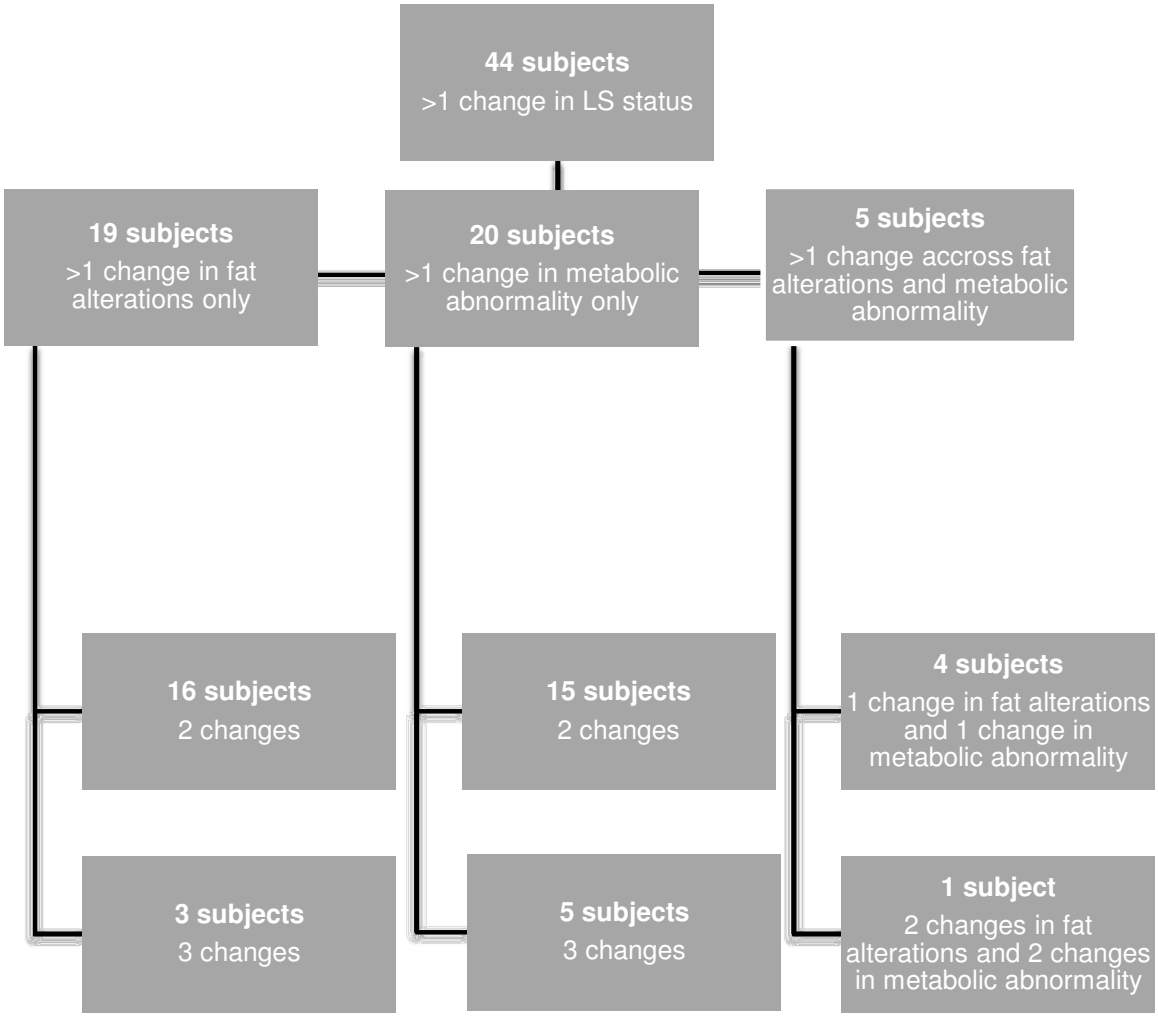
Incident fat alterations – face: *n* = 27, arms: *n* = 31, legs: *n* = 33, buttocks: *n* =43, trunk: *n* = 28, neck: *n* = 22, and breast: *n* = 30. Reversed fat alterations - face: *n* = 29, arms: *n* = 31, legs: *n* = 30, buttocks: *n* = 26, trunk: *n* = 31, neck: *n* = 20, and breast: *n* = 25. See Appendix F: Table F-3 for details of specific person time at risk

Figure 6-6: Rates of incidence or complete reversal of specific metabolic abnormality



Incident metabolic abnormality – hypercholesterolemia: *n* = 27, fasting hypertriglyceridemia: *n* = 29. Reversed metabolic abnormality - hypercholesterolemia: *n* = 37, fasting hypertriglyceridemia: *n* = 16. Total follow-up: 1318.65 person-years. See Appendix F: Table F3 for details of specific person time at risk

Figure 6-7: Subjects at least one occurrence of lipodystrophy syndrome incidence and at least one occurrence of lipodystrophy syndrome symptoms complete reversal over follow-up



6.3.6 Probability of incident lipodystrophy syndrome outcomes

The probabilities of incident fat alterations, metabolic abnormality and LS among subjects without LS at recruitment were assessed by plotting Kaplan Meier plots: plots for LS, body fat alterations or metabolic abnormality are reproduced in the Section F.5 in Appendix F. Log-rank testing was used to explore differences in survival function between groups for each outcome: *p*–values are presented in Table 6-7.

There was no consistent pattern in the incidence of LS symptoms by maximum clinical status: while the probability of incidence of fat alterations (Appendix F: Figure F-10a) was highest

amongst subjects without a history of symptomatic disease for much of the follow-up, this was less clear with respect to the incidence of metabolic abnormality (Appendix F: Figure F-10b) or LS (Appendix F: Figure F-9a). Indeed, significant differences were seen between subjects of differing maximum clinical status with respect to body fat alterations (p = 0.034): at 4 years follow-up, the probability for LS was approximately 25% in subjects with a history of moderate clinical symptoms while the estimated probability of LS in either subjects with no symptoms or severe symptoms was 60%. Differences in maximum clinical status were non-significant for both metabolic abnormality and LS. While the incidence of fat alterations or metabolic abnormality varied with history of immunosuppression, subjects with past nadir moderate immunosuppression had a higher probability of incident LS for the first 3 years of follow-up (Appendix F: Figure F-9b). However, there was no significant difference in the survival between subjects of differing immunosuppression for any outcome ($p \geq 0.183$).

The probability of incident metabolic abnormality or LS was higher in subjects who were symptomatic for HIV at recruitment (Appendix F: Figure F-13b and Figure F-11a respectively). The probability of incident body fat alterations was highest in subjects who were asymptomatic at recruitment (Appendix F: Figure F-13a). Indeed, significant differences in survival function between groups was seen only for body fat alterations (p = 0.002). In contrast, similar patterns in incidence of LS symptoms were seen across subjects with differing CDC-defined immunosuppression at recruitment. During early follow-up, subjects with the most severe immunosuppression at recruitment had the highest probability of LS (Appendix F: Figure F-11b): the probability of LS was 100% in subjects with severe immunosuppression at 2 years of follow-up, compared with approximately 80% for subjects with either no or moderate immunosuppression. During later follow-up subjects with moderate immunosuppression or no immunosuppression had the highest probability, i.e. approximately 50% for no/moderate immunosuppression at 4 years of follow-up compared to 0% probability of LS with severe immunosuppression. There was no evidence of differences in survival function between groups of differing recruitment immunosuppression with any outcome ($p \geq 0.117$).

The probability of two LS outcomes (incident metabolic abnormality and incident LS) was highest in subjects who had yet to undergo puberty at recruitment, followed by those who had completed puberty (Appendix F: Figure F-16b and Figure F-15a in this chapter). However the opposite was seen with incident fat alterations: the highest probability was in subjects with who had completed puberty (approximately 70% compared to 60% in pre-pubescent children, and 45% in post-pubertal adolescents: Appendix F Figure F-16a). While significant differences were seen in survival function between groups of different Tanner score by LS status (p = 0.011), differences were not statistically significant for body fat alterations (p = 0.974), and metabolic abnormality (p = 0.634).

Use of NNRTI at recruitment was associated with a greater probability of incident metabolic abnormality (Appendix F: Figure F-17b) and LS (Figure F-15b) during follow-up compared to no-use at recruitment: these differences were only significant for body fat alterations (p = 0.012), where the probability at four years of follow-up amongst subjects on NNRTI at recruitment was almost 85%, but was approximately 60% in subjects not on this class of ART. The probabilities of incident body fat alterations and metabolic abnormality was lower amongst subjects using PI at recruitment, compared to subjects not using this class of ART (Appendix F: Figure F-19): while this pattern was also true for incident LS, it was not as obvious (Figure F-18). Significant differences in survival function were not seen with recruitment PI use for any LS outcome ($p \geq$ 0.732).

A similar pattern of incidence of fat alterations, metabolic abnormality, or LS, was seen with use of HAART at recruitment (Appendix F: Figure F-20, and Figure 6-18b in this chapter): in early follow-up the probability was greater among subjects on HAART at recruitment, but after approximately 2 years of follow-up the converse was true with the probability being comparatively higher amongst subjects not on HAART at recruitment. At 4-years of follow-up the probability of incident body fat alterations amongst subjects not on HAART was approximately 70% compared to 25% in those subjects on HAART at recruitment (Figure F-20a): this difference was statistically significant (p = 0.003). However no significant difference were seen either incident metabolic abnormality (p = 0.054), nor incident LS (p = 0.621).

When investigating specific ART regimen used at recruitment, no incident body fat alteration during follow-up were seen in subjects who were on triple class therapy at recruitment (Appendix F: Figure F-22a). The highest probability of incident body fat alterations was seen in subjects on NNRTI-based HAART at recruitment, followed by those on PI-based HAART, i.e. approximately 70% and 50% at 4 years follow-up. In contrast, for the majority of follow-up, the highest probability of incident metabolic abnormality was seen with NNRTI-based HAART, followed by NRTI-mono-therapy, PI-based HAART and triple class therapy (Figure F-22b). For much of the follow-up period, NNRTI-based HAART was associated with the greatest probability of incident LS, PI-based HAART and NRTI mono-therapy (Figure F-21a). However, at 3.5 years of follow-up, the probability of LS was highest amongst subjects on NRTI mono-therapy at recruitment. Significant differences in survival function were seen between subjects on different ART regimens at recruitment for body fat alterations (p = 0.003), but not for either metabolic abnormality or LS ($p \geq$ 0.118).

Significant differences were seen in the survival function between subjects who were on zidovudine at recruitment and those not on this drug for body fat alterations (p = 0.009) and metabolic abnormality (p = 0.034), as illustrated in Table 6 7. Furthermore there were significant differences in the survival function for incident LS between subjects who had detectable viral load during follow-up compared to those who did not for both LS (p = 0.040, Figure F-24a), and

body fat alterations (p <0.001, Appendix F: Figure F-26a). Significant differences were also seen in the survival function for body fat alterations (p < 0.001) and for metabolic abnormality (p = 0.040) between subjects who had experienced any CDC-defined clinical symptoms over follow-up (Appendix F: Figure F-27). Similarly, the survival functions for LS in subjects who had experienced CDC-defined immunosuppression over follow-up and those who had not were significantly different (p = 0.046) as illustrated in Figure F-25.

Table 6-7: Log-rank tests comparing survival functions by lipodystrophy outcome

	p –value for log-rank test		
	Lipodystrophy syndrome	**Body fat alterations**	**Metabolic abnormality**
Recruitment age	0.036	0.069	0.581
Gender	0.952	0.918	0.860
Maximum CDC clinical stage	0.284	0.034	0.280
Nadir CDC-defined immunosuppression	0.770	0.183	0.507
Recruitment CDC-defined clinical status	0.365	0.002	0.085
Recruitment immune status	0.620	0.117	0.297
Recruitment Tanner score	0.011	0.974	0.634
Recruitment lamivudine	0.292	0.244	0.254
Recruitment stavudine	0.965	0.147	0.406
Recruitment efavirenz	0.678	0.432	0.835
Recruitment tenofovir	0.730	0.671	0.989
Recruitment zidovudine	0.868	0.009	0.034
Recruitment ritonavir booster	0.193	0.782	0.556
Recruitment PI use	0.732	0.837	0.975
Recruitment NRTI use	0.436	0.706	0.191
Recruitment NNRTI use	0.974	0.012	0.155
Recruitment HAART	0.621	0.003	0.054
Recruitment ART regimen	0.821	0.003	0.118
Recruitment duration of ART use	0.635	0.638	0.698
Recruitment BMI	0.476	0.179	0.099
Any detectable virus during follow-up	0.040	<0.001	0.143
Any clinical symptoms during follow-up	0.597	<0.001	0.040
Any severe (CDC class C) clinical symptom during follow-up	0.225	0.391	0.672
Any immunosuppression during follow-up	0.046	0.964	0.939
Any severe immunosuppression during follow-up	<0.001	0.009	0.284

6.3.7 Factors associated with the incidence of lipodystrophy syndrome outcomes

Body fat alterations

While there was no statistically significant association between recruitment factors and incident fat alterations in univariable Cox proportional hazard models, detectable viral load and use of PI were associated with a non-significant increased risk (Table 6-8). Any detectable viral load and any CDC-clinical symptoms during follow-up were both associated with a non-significant increased risk of incident body fat alterations. Any CDC-defined immunosuppression was associated with a non-significant decrease in risk. Furthermore, female sex and maximum clinical symptoms was associated with a non-significant increase in risk.

Table 6-8: Univariable Cox regression models for incidence of lipodystrophy syndrome outcomes

		Body fat alterations			Metabolic abnormality			Lipodystrophy syndrome		
		Hazard ratio	95% confidence interval	*p* - value	Hazard ratio	95% confidence interval	*p* - value	Hazard ratio	95% confidence interval	*p*- value
Age (years)*	**2-11**	1			1			1		
	12-18	1.37	(0.76, 2.48)	0.300	1.30	(0.62, 2.76)	0.488	2.26	(1.14, 4.48)	0.019
Duration of	**<2.5**	1			1			1		
ART (years)*	**2.5-4.9**	0.89	(0.34, 2.34)	0.818	0.57	(0.17, 1.91)	0.364	1.01	(0.37, 2.81)	0.978
	5.0-7.4	1.57	(0.55, 4.46)	0.397	0.57	(0.15, 2.17)	0.430	0.59	(0.17, 2.03)	0.405
	7.5-9.9	1.96	(0.82, 4.70)	0.132	0.65	(0.20, 2.17)	0.487	1.03	(0.37, 2.86)	0.953
	≥10.0	0.69	(0.19, 2.57)	0.582	0.96	(0.33, 2.78)	0.947	1.40	(0.51, 3.87)	0.517
Sex	**Male**	1			1			1		
	Female	1.58	(0.84, 2.95)	0.156	0.89	(0.43, 1.85)	0.757	1.11	(0.59, 2.10)	0.744
Ethnicity	**Black**	1			1			1		
	White	0.94	(0.48, 1.83)	0.858	0.38	(0.17, 0.88)	0.024	0.21	(0.10, 0.45)	<0.001
	Other	1.26	(0.28, 5.69)	0.762	0.53	(0.07, 4.17)	0.547	0.37	(0.05, 2.74)	0.328
Tanner score	**I**	1			1			1		
for puberty	**II-IV**	1.31	(0.62, 2.77)	0.477	3.52	(1.17, 10.62)	0.026	5.25	(1.96, 14.01)	0.001
	V	0.98	(0.38, 2.55)	0.973	2.35	(0.69, 8.02)	0.174	3.74	(1.25, 11.21)	0.018
Detectable	**<50 copies/ml**	1			1			1		
viral load*	**≥50 copies/ml**	1.59	(0.38, 6.76)	0.523	1.43	(0.34, 6.11)	0.626	4.25	(0.58, 31.15)	0.15
Detectable	**No**	1			1			-		
viral load at any time**	**Yes**	1.20	(0.28, 5.07)	0.809	2.43	(0.33, 18.04)	0.385	-	-	-
CDC-defined	**N/A**	1			1			1		
clinical	**B**	0.82	(0.29, 2.36)	0.715	0.52	(0.07, 3.91)	0.529	0.57	(0.08, 4.21)	0.580
status *	**C**	2.53	(0.59, 10.80)	0.209	-	-	-	-	-	-
Maximum	**N/A**	1			1			1		
CDC-defined	**B**	1.93	(0.88, 4.22)	0.101	1.01	(0.41, 2.49)	0.978	1.28	(0.58, 2.84)	0.536
clinical status	**C**	1.85	(0.81, 4.22)	0.146	0.98	(0.37, 2.60)	0.970	0.89	(0.37, 2.15)	0.793
Any CDC-	**No**	1			1			1		
defined	**Yes**	1.22	(0.65, 2.31)	0.535	0.60	(0.25, 1.46)	0.264	0.86	(0.42, 1.78)	0.694
symptoms**										

		Body fat alterations			Metabolic abnormality			Lipodystrophy syndrome		
		Hazard ratio	95% confidence interval	*p* - value	Hazard ratio	95% confidence interval	*p* - value	Hazard ratio	95% confidence interval	*p*- value
CDC-defined immuno-suppression*	**1**	1			1			1		
	2	0.75	(0.37, 1.50)	0.418	1.10	(0.48, 2.50)	0.824	0.91	(0.43, 1.92)	0.807
	3	1.03	(0.14, 7.67)	0.974	1.53	(0.20, 11.40)	0.681	3.07	(0.92, 10.26)	0.068
Nadir CDC-defined immuno-suppression	**1**	1			1			1		
	2	1.69	(0.82, 3.51)	0.155	1.56	(0.67, 3.64)	0.306	0.86	(0.40, 1.85)	0.703
	3	1.05	(0.48, 2.31)	0.901	0.75	(0.25, 2.29)	0.614	1.13	(0.50, 2.53)	0.773
Any CDC-defined immuno-suppression**	**No**	1			1			1		
	Yes	0.61	(0.33, 1.14)	0.123	0.93	(0.45, 1.93)	0.842	1.17	(0.62, 2.21)	0.619
Body Mass index*	**Healthy weight**	1			1			1		
	Severely underweight	0.59	(0.25, 1.36)	0.212	0.60	(0.17, 2.11)	0.430	0.42	(0.15, 1.27)	0.127
	Underweight	0.53	(0.24, 1.18)	0.123	0.56	(0.20, 1.57)	0.273	0.78	(0.37, 1.64)	0.514
	Overweight	0.66	(0.09, 4.93)	0.683	3.45	(1.32, 9.02)	0.012	2.68	(0.89, 8.04)	0.079
PI*	**No use**	1			1			1		
	Use	1.13	(0.61, 2.09)	0.693	2.72	(1.15, 6.44)	0.023	1.15	(0.58, 2.26)	0.690
NNRTI*	**No use**	1			1			1		
	Use	1.02	(0.50, 2.07)	0.959	0.44	(0.16, 1.19)	0.108	0.84	(0.38, 1.84)	0.657

CDC-defined immune status - stage 1: no immunosuppression, stage 2: moderate immunosuppression, stage 3: severe immunosuppression. CDC-defined clinical status - N + A: no symptoms/asymptomatic, B: moderate symptoms, C: severe symptoms. *Status at recruitment **Occurring over follow-up period.

None of the postulated risk factors (Table 6-2) were statistically significant in the multivariable model examining the association between recruitment factors and incident body fat alterations: consequently the final model only included age and BMI at recruitment, and the random effect of clinical site (Table 6-9). In the multivariable model including ART regimen at recruitment, NRTI use was associated with a non-significant decreased risk (Adjusted hazard ratio, AHR: 0.40, 95% CI: 0.13, 1.24), and NNRTI-based HAART with a non-significant increased risk (AHR: 1.13, 95% CI: 0.49, 2.61) compared to PI-based HAART (Appendix F: Table F-4).

Table 6-9: Final multivariable Cox regression model for incident body fat alterations: covariates at recruitment (*n* = 194)

		Adjusted hazard ratio	95% confidence interval	*p* – value
Age (years)*	**2-11**	1		
	12-18	1.35	(0.67, 2.74)	0.400
Duration of ART (years)*	**<2.5**	1		
	2.5-4.9	0.95	(0.36, 2.51)	0.919
	5.0-7.4	1.62	(0.57, 4.61)	0.366
	7.5-9.9	1.71	(0.69. 4.21)	0.244
	≥10.0	0.60	(0.15, 2.31)	0.454

Multivariable model contains a random effect for clinical site of treatment. *Status at recruitment. Therneau-Grambsch global test for proportional hazards: *p* = 0.8841

In contrast to the model including recruitment covariates, in the final multivariable model including time-updated covariates during follow-up, any immunosuppression during the follow-up period was associated with a significant decrease in risk of incident body fat alterations (AHR: 0.41, 95% CI: 0.19, 0.91), as illustrated in Table 6-10: no other factor remained statistically significant in this model.

Table 6-10: Final multivariable Cox regression models for incident body fat alterations: covariates during follow-up (*n* = 194)

		Adjusted hazard ratio	95% confidence interval	*p* – value
Age (years)*	**2-11**	1		
	12-18	1.84	(0.86, 3.94)	0.116
Duration of ART (years)*	**<2.5**	1		
	2.5-4.9	1.02	(0.38, 2.79)	0.960
	5.0-7.4	1.86	(0.63, 5.47)	0.261
	7.5-9.9	2.00	(0.81, 4.96)	0.135
	≥10.0	-	-	-
Immuno-suppression**	**No immunosuppression**	1		
	Immunosuppression	0.41	(0.19, 0.91)	0.027

Multivariable model contains a random effect for clinical site of treatment. *Status at recruitment. **Time updated covariate. Therneau-Grambsch global test for proportional hazards: *p* = 0.6726

Metabolic abnormality

A significant reduced risk of incident metabolic abnormality was found to be associated with White ethnicity (HR: 0.38, 95% CI: 0.17, 0.88) in unadjusted Cox modelling (Table 6-9). A significantly increased risk seen in subjects who were undergoing puberty (HR: 3.52, 95% CI: 1.17, 10.62), compared to those who were yet to begin puberty. Although an increased risk was also seen in subjects who had completed puberty, this was non-significant (p = 0.174). Furthermore an overweight BMI (25.0m/kg^2 ≤ BMI < 30.025.0m/kg^2) was also a significant risk factor for incident metabolic abnormality (HR: 3.45. 95% CI: 1.32, 9.02), compared to subjects who had a healthy BMI at recruitment (Table 6-8). Use of PIs at recruitment was associated with a significant increased risk (HR: 2.72, 95% CI: 1.15, 6.44), while use of NNRTI was associated with a non-significant (p = 0.108) reduced risk (HR: 0.44, 95% CI: 0.16, 1.19).

Both overweight BMI (AHR: 4.83, 95% CI: 1.38, 16.91), and recruitment use of PI (AHR: 3.56, 95% CI: 1.18, 10.80) were significant and independent risk factors for incident metabolic abnormality in the final multivariable model including recruitment risk factors (Table 6-11). This is supported by the multivariable model including ART regimen at recruitment (Appendix F: Table F-4), where NNRTI-based HAART was associated with a reduced risk (AHR: 0.27, 95% CI: 0.08, 0.95), compared to PI-based HAART.

Table 6-11: Final multivariable Cox regression models for incident metabolic alterations: covariates at recruitment (n = 401)

		Adjusted hazard ratio	95% confidence interval	p – value
Age (years)*	**2-11**	1		
	12-18	0.97	(0.35, 2.71)	0.951
Duration of ART (years)*	**<2.5**	1		
	2.5-4.9	0.50	(0.14, 1.81)	0.293
	5.0-7.4	0.48	(0.12, 1.90)	0.297
	7.5-9.9	0.58	(0.16, 2.09)	0.401
	≥10.0	0.47	(0.11, 2.00)	0.307
Body Mass index*	**Healthy weight**	1		
	Severely underweight	0.48	(0.09, 2.55)	0.390
	Underweight	0.92	(0.30, 2.81)	0.884
	Overweight	4.83	(1.38, 16.91)	0.014
PI*	**No use**	1		
	Use	3.56	(1.18, 10.80)	0.025

Multivariable model contains a random effect for clinical site of treatment. *Status at recruitment. Therneau-Grambsch global test for proportional hazards: p = 0.6684

Furthermore, recruitment PI remained statistically significant in the final multivariable model including risk factors during follow-up (Table 6-12) being associated with a 4-fold increase in risk (AHR: 4.44, 95% CI: 1.40, 14.12). Moreover, recruitment obese BMI remained a significant risk model in this model. However, any CDC-defined immunosuppression during the follow-up period was associated with a significant and independent decrease in risk of incident metabolic abnormality (AHR: 0.34, 95% CI: 0.12, 0.98).

Table 6-12: Final multivariable Cox regression models for incident metabolic alterations: covariates during follow-up (*n* = 401)

		Adjusted hazard ratio	95% confidence interval	*p* – value
Age (years)*	**2-11**	1		
	12-18	1.06	(0.38, 2.91)	0.915
Duration of ART (years)*	**<2.5**	1		
	2.5-4.9	0.61	(0.17, 2.18)	0.450
	5.0-7.4	0.43	(0.11, 1.72)	0.232
	7.5-9.9	0.63	(0.17, 2.26)	0.476
	≥10.0	0.56	(0.13, 2.43)	0.443
Body Mass index*	**Healthy weight**	1		
	Severely underweight	0.47	(0.09, 2.51)	0.381
	Underweight	0.91	(0.29, 2.79)	0.862
	Overweight	7.21	(1.91, 27.27)	0.004
PI*	**No use**	1		
	Use	4.44	(1.40, 14.12)	0.012
Immuno-suppression**	**No immunosuppression**	1		
	Immunosuppression	0.34	(0.12, 0.98)	0.046

***Status at recruitment. **Time updated covariate. Therneau-Grambsch global test for proportional hazards: *p* = 0.8547**

Lipodystrophy syndrome

In univariable analyses, subjects who were of White ethnicity had a statistically significant reduced risk of incident LS (HR: 0.21, 95% CI: 0.10, 0.45) (Table 6-8). Children and adolescents aged 12-18 years at recruitment had a significant increase in risk that was over two-fold that for 2-11 year olds (HR: 2.26, 95% CI: 1.14, 4.48). Indeed, subjects who were undergoing puberty (HR: 5.25, 95% CI: 1.96, 14.01), or had completed puberty (HR: 3.74, 95% CI: 1.25, 11.21), had a significantly increased risk compared to subjects who had yet to commence puberty.

In adjusted analyses, both White ethnicity and maximum CDC-defined clinical condition were significant risk factors for incident LS (Table 6-13). However, while White ethnicity was associated with a decreased risk (AHR: 0.15, 95% CI: 0.05, 0.38), maximum clinical status B

was associated with increased risk (AHR: 2.56, 95% CI: 1.00, 6.54). Neither use of PIs nor NNRTIs at recruitment were significant risk factors for incident LS. Indeed, no ART regimen was significantly associated with incident LS in the multivariable Cox modelling (Appendix F: Table F-4).

Table 6-13: Final multivariable Cox regression models for incident lipodystrophy syndrome: covariates during follow-up

		Adjusted hazard ratio	95% confidence interval	*p* – value
Age(years)*	**2-11**	1		
	12-18	1.70	(0.68, 4.15)	0.243
Duration of ART (years)*	**<2.5**	1		
	2.5-4.9	0.49	(0.16, 1.51)	0.218
	5.0-7.4	0.44	(0.11, 1.70)	0.234
	7.5-9.9	1.48	(0.49, 4.46)	0.489
	≥10.0	0.53	(0.15, 1.89)	0.331
Ethnicity	**Black**	1		
	White	0.15	(0.05, 0.38)	<0.001
	Other	0.69	(0.08, 5.54)	0.724
Maximum CDC-defined clinical status	**N/A**	1		
	B	2.56	(1.00, 6.54)	0.049
	C	0.83	(0.31, 2.26)	0.720

CDC-defined clinical status - N + A: no symptoms/asymptomatic, B: moderate symptoms, C: severe symptoms. Multivariable model contains a random effect for clinical site of treatment. *Status at recruitment. Therneau-Grambsch global test for proportional hazards: *p* = 0.8430

Survival analysis of follow-up factors associated with incidence of LS did not result in a Cox model which converged.

Proportional hazards assumption in Cox regression models

Both examination of Schoenfeld residual plots with follow-up time, and performing GLOBAL significant tests of the multivariable models showed that the proportional hazards assumptions in explanatory variables had been met. Results from the GLOBAL tests are included in the Appendix F: Section F.7.

6.3.8 Progression and/or regression of lipodystrophy syndrome symptoms

At recruitment, 235 subjects had LS: of these subjects 221 had prospective data collected from which assessment regarding progression or regression of these symptoms could be made: data was available for 134 subjects who had body fat alterations alone, 49 subjects who had metabolic abnormality alone, and 38 subjects who had both (Table 6-14). In each of these

groups, change in severity of body fat alterations symptoms was similar: ~75% experienced regression alone, and ~25% experienced both progression and regression over follow-up. However, differences were seen in change in metabolic symptoms over time between the three groups. Children with initial body fat alterations only most frequently experienced progression alone (n = 49, 38.9%), those with metabolic abnormality only, or both at recruitment most frequently experienced regression alone (n = 15, 34.1% and n = 13, 35.1% respectively).

All these 221 subjects experienced both progression and regression of symptoms during the follow up period. The most common manifestation was progression of metabolic abnormality symptoms with accompanying regression of body fat alteration symptoms (41.7%, n = 95), followed by regression of both fat alteration and metabolic abnormality symptoms (36.4%, n = 83) as illustrated in Figure 6-8.

Change in severity of body fat alteration symptoms

Over 70% (n/N = 143/201) of subjects with recruitment LS experienced regression with no progression of body fat alterations, while 28.9% (n/N = 58/201) had both progression and regression (Figure 6-9). However 27 subjects were missing data on change in symptoms of body fat alterations. No significant difference was seen in either median age, or median duration of ART use between subjects with only regression of body fat alteration compared to those with both progression and regression ($p \geq 0.410$).

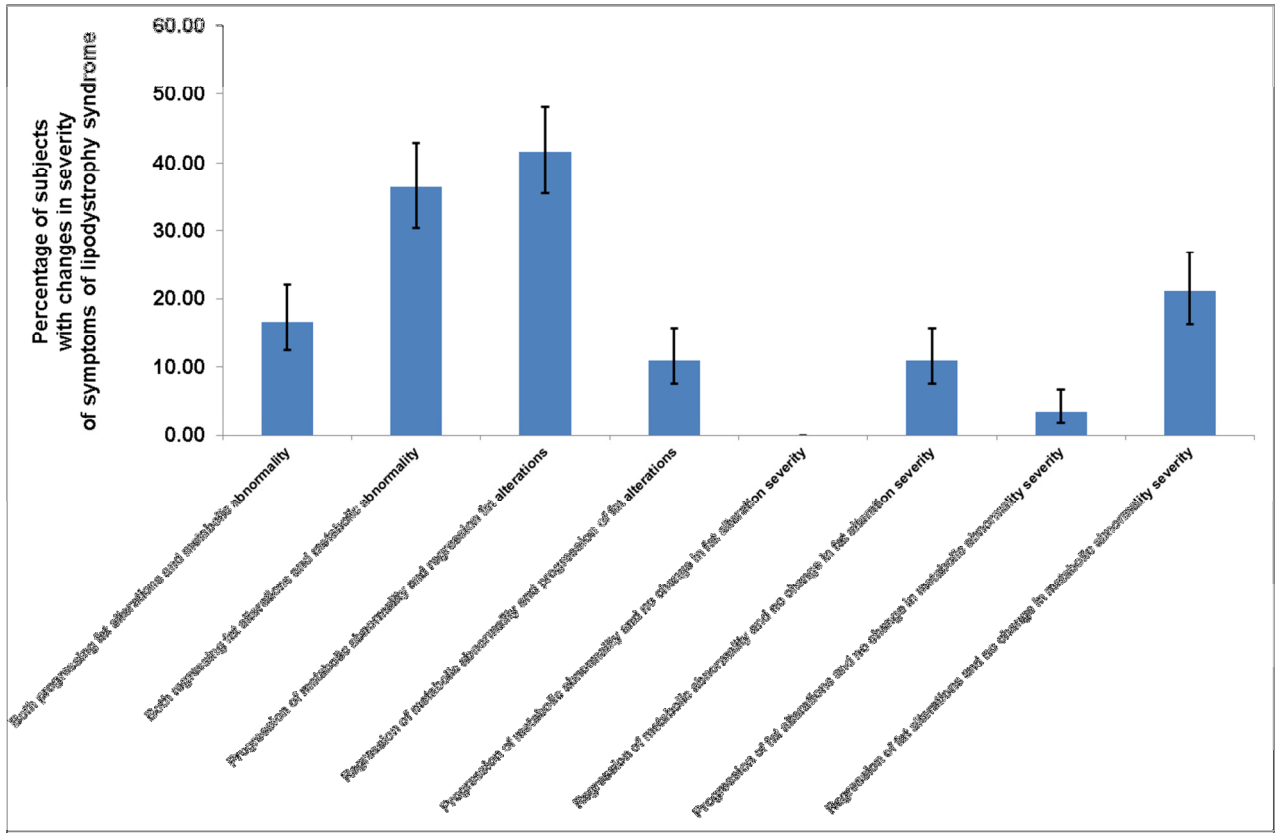
Change in severity of metabolic abnormality symptoms

The proportion of subjects who had no change in the severity of metabolic abnormality, or who had experienced regression without progression over follow up was similar: 24.8% (n/N = 53/214), and 24.3% (n/N = 52/214) respectively (Figure 6-9b). Almost 30% (n/N = 64/214) of subjects who had LS at recruitment had progression of metabolic abnormality during follow-up, while 21.0% (n/N = 45/214) experienced both progression and regression. The number of subjects who were missing data on change in metabolic symptoms over the follow-up period was 14. There was no significant difference in either age or duration of ART use at recruitment between subjects with differing metabolic abnormality symptom profiles over follow-up.

Table 6-14: Specific changes in symptom severity over follow-up by initial lipodystrophy status (*n* = 221)

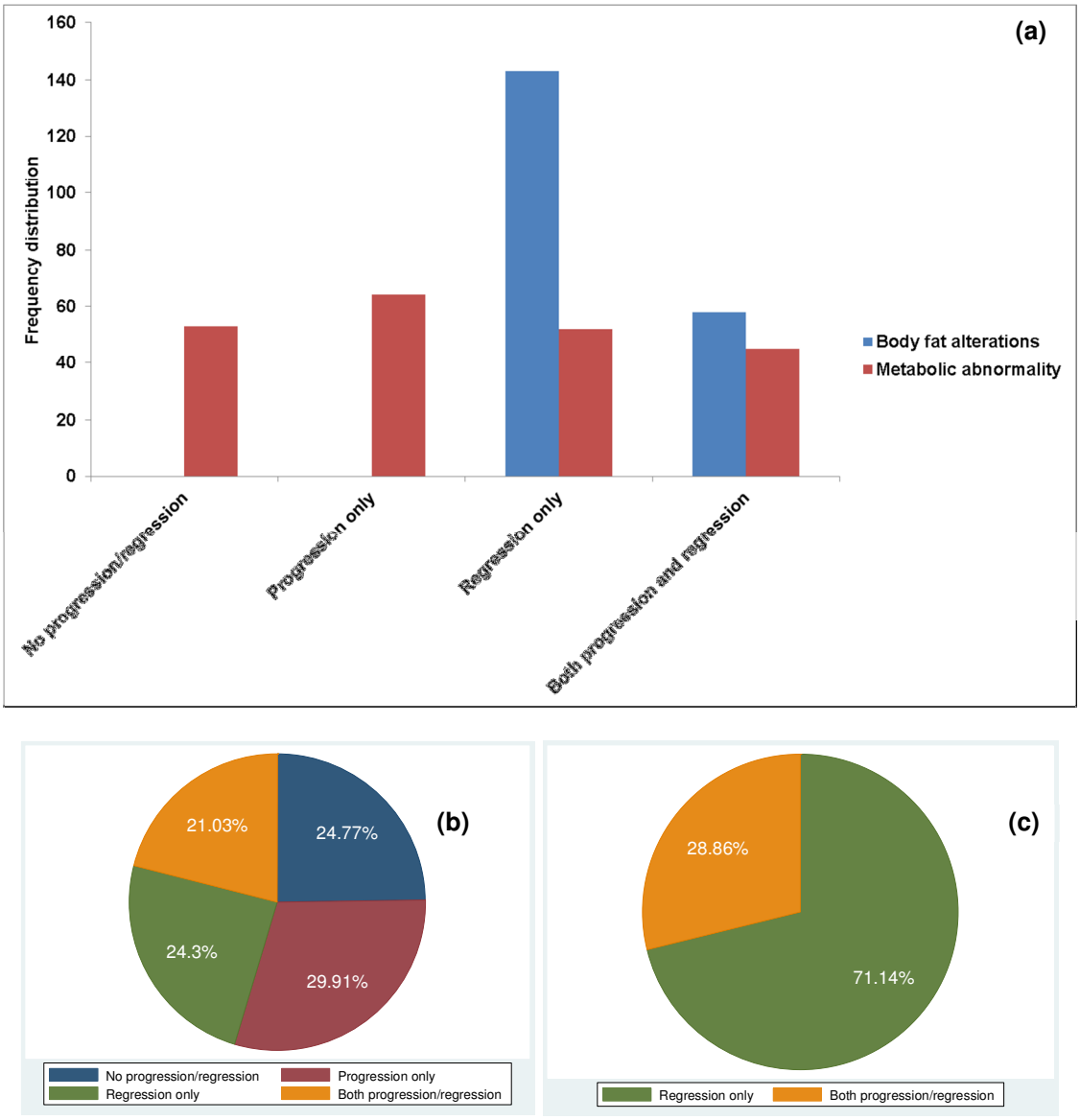
Metabolic abnormality					*Body fat alterations*			
Both progression and regression	**Progression only**	**Regression only**	**Neither progression nor regression**	**INITIAL DIAGNOSES**	**Neither progression nor regression**	**Regression only**	**Progression only**	**Both progression and regression**
25 (19.8%)	49 (38.9%)	22 (17.5%)	30 (23.8%)	**Body fat alterations only (*n* = 134)**	0	79 (67.0%)	0	39 (33.1%)
11 (25.0%)	9 (20.5%)	15 (34.1%)	9 (20.5%)	**Metabolic abnormality only (*n* = 49)**	0	31 (75.6%)	0	10 (24.4%)
9 (24.3%)	6 (16.2%)	13 (35.1%)	9 (24.3%)	**Both body fat alterations and metabolic abnormality (*n* = 38)**	0	27 (75.0%)	0	9 (25.0%)

Figure 6-8: Manifestation of progression/regression of symptoms over follow-up in subjects with lipodystrophy syndrome at recruitment (*n* = 221*)



***221 of the 235 subjects with LS at recruitment had data available on progression/regression of symptoms over follow-up**

Figure 6-9: Distribution of progression and regression of symptoms of lipodystrophy syndrome: as (a) frequency distribution, and percentage distribution of (b) body fat alterations; *n* = 201, and (c) metabolic abnormality; *n* = 214



Missing data: body fat alterations; 27, and metabolic abnormality; 14

Associated factors

Subject characteristics were examined in relation to 4 symptom-change outcomes with respect to body fat alterations and metabolic abnormality: no change, progression only, regression only, both progression and regression

No significant associations were seen with any subject characteristic and changes in symptom severity of either body fat alterations (Table 6-15) or metabolic abnormality (Table 6-16).

Although the difference was statistically non-significant, 97% of subjects with regression of body fat alterations symptoms only had detectable viral load over follow-up, while this percentage was 91% in subjects with both progression and regression.

Table 6-15: Characteristics of subjects with changes in severity of symptoms of body fat alterations during follow-up (*n* = 201)

		No progression/ regression	Progression only	Regression only	Both progression and regression	*p*-value
Recruitment clinical condition	**N/A**	0	0	114 (90.48)	45 (93.75)	
	B	0	0	10 (7.94)	2 (4.17)	
	C	0	0	2 (1.59)	1 (2.08)	0.668
Recruitment immuno-suppression	**Stage 1**	0	0	116 (81.12)	47 (81.03)	
	Stage 2	0	0	22 (15.38)	10 (17.24)	
	Stage 3	0	0	5(3.50)	1 (1.72)	0.770
Maximum clinical condition	**N/A**	0	0	37 (27.82)	13 (24.53)	
	B	0	0	57 (42.86)	22 (41.51)	
	C	0	0	39 (29.32)	18 (33.96)	0.805
Nadir immune status	**Stage 1**	0	0	36 (25.17)	14 (24.14)	
	Stage 2	0	0	64(44.76)	26 (44.83)	
	Stage 3	0	0	43 (30.07)	18 (31.03)	0.985
Any clinical symptoms*	**No symptoms**	0	0	3(3.03)	5 (9.26)	
	Symptoms	0	0	96 (96.97)	49 (90.74)	0.098
Any immuno-suppression*	**None**	0	0	99 (69.23)	44 (30.77)	
	Immunosuppression	0	0	40 (68.97)	18 (31.03)	0.971
Recruitment viral load	**Undetectable**	0	0	21 (14.69)	9 (15.52)	
	Detectable	0	0	122 (85.31)	49 (84.48)	0.881
Any detectable viral load*	**No detection**	0	0	4 (7.14)	5 (14.71)	
	Detected	0	0	52 (92.86)	29 (85.29)	0.246
Recruitment antiretroviral regiment	**NRTI mono-therapy**	0	0	9 (6.98)	3 (5.77)	
	PI-based HAART	0	0	75 (58.14)	31(59.62)	
	NNRTI-based HAART	0	0	37 (28.68)	17 (32.69)	
	Triple class therapy	0	0	8 (6.20)	1 (1.92)	0.646
Any change in ART regimen*	**No change**	0	0	108 (80.60)	41 (73.21)	
	Change	0	0	26 (19.40)	15 (26.79)	0.259

All subjects had lipodystrophy syndrome at recruitment. CDC-defined immune status - stage 1: no immunosuppression, stage 2: moderate immunosuppression, stage 3: severe immunosuppression. CDC-defined clinical status - N + A: no symptoms/asymptomatic, B: moderate symptoms, C: severe symptoms. Detectable viral load defined as >50 copies/mL. *Over follow-up

Non-significant differences were also seen between some patient characteristics and changes in metabolic abnormality symptoms (Table 6-16). Approximately 86% of subjects either with (i) no changes in metabolic abnormality severity or (ii) regression of symptoms only had no immunosuppression at recruitment: this proportion was approximately 75% for subjects with either progression of symptoms only, or those with both progression and regression. Almost one quarter of subjects who experienced both progression and regression of metabolic abnormality had undetectable viral load, with this proportion being less than 15% for other symptom-change outcomes. Over half of subjects with each symptom-change outcome were on PI-based HAART at recruitment: 55-57% of subjects with either progression only or regression only, 65% of subjects with no change in severity of metabolic abnormality, and 46% in subjects with both progression and regression of symptoms.

Table 6-16: Characteristics of subjects with changes on severity of symptoms of metabolic abnormality (*n* = 214)

		No progression/ regression	Progression only	Regression only	Both progression and regression	*p*-value
Recruitment clinical condition	**N/A**	40 (86.96)	53 (92.98)	39 (88.64)	37 (97.37)	
	B	4 (8.70)	4 (7.02)	4 (9.09)	0	
	C	2 (4.35)	0	1 (2.27)	1 (2.63)	0.436
Recruitment immuno-suppression	**Stage 1**	46 (86.79)	48 (75.00)	45 (86.54)	35 (77.78)	
	Stage 2	7 (13.21)	13 (20.31)	6 (11.54)	9 (20.00)	
	Stage 3	0	3 (4.69)	1 (1.92)	1 (2.22)	0.471
Maximum clinical condition	**N/A**	13 (27.66)	18 (29.03)	15 (30.00)	12 (30.77)	
	B	19 (40.43)	21 (33.87)	22 (44.00)	82 (41.41)	
	C	15 (31.91)	23 (37.10)	13 (26.00)	58 (29.29)	0.513
Nadir immune status	**Stage 1**	15 (28.30)	17 (26.56)	13 (25.00)	11 (24.44)	
	Stage 2	21 (39.62)	30 (46.88)	21 (40.38)	22 (48.89)	
	Stage 3	17 (32.08)	17 (26.56)	18 (34.62)	12 (26.67)	0.938
Any clinical symptoms*	**No symptoms**	3 (10.34)	5 (8.62)	2 (4.76)	6 (15.38)	
	Symptoms	26 (89.66)	53 (91.38)	40 (95.24)	33 (84.62)	0.434
Any immuno-suppression*	**None**	39 (73.58)	42 (65.63)	35 (67.31)	29 (64.44)	
	Immunosuppression	14 (26.42)	22 (34.38)	17 (32.69)	16 (35.56)	0.756
Recruitment viral load	**Undetectable**	7 (13.21)	9 (14.06)	6 (11.54)	11 (24.44)	
	Detectable	46 (86.79)	55 (85.94)	46 (88.46)	34 (75.56)	0.296
Any detectable viral load*	**No detection**	4 (22.22)	5 (14.29)	3 (11.11)	7 (25.00)	
	Detected	14 (77.78)	30 (85.71)	24 (88.89)	21 (75.00)	0.499
Recruitment antiretroviral regiment	**NRTI mono-therapy**	3 (6.52)	7 (11.67)	0	2 (4.88)	
	PI-based HAART	30 (65.22)	33 (55.00)	27 (56.25)	19 (46.34)	
	NNRTI-based HAART	12 (26.09)	19 (31.67)	17 (35.42)	16.(39.02)	
	Triple class therapy	1 (2.17)	1 (1.67)	4 (8.33)	4 (9.76)	0.140
Any change in ART regimen*	**No change**	39 (76.47)	48 (77.42)	42 (85.71	30 (69.77)	
	Change	12 (23.53)	14 (22.58)	7 (14.29)	13 (30.23)	0.333

All subjects had lipodystrophy syndrome at recruitment. CDC-defined immune status - stage 1: no immunosuppression, stage 2: moderate immunosuppression, stage 3: severe immunosuppression. CDC-defined clinical status - N + A: no symptoms/asymptomatic, B: moderate symptoms, C: severe symptoms. Detectable viral load defined as >50 copies/mL. *Over follow-up

6.4 Key points

- The median duration of follow-up of subjects was 4.19 years (IQR CI: 3.00, 3.97) (total person years = 1318.65 years) with up to 4 data collections points for each subject.

- There were increases in the prevalence of detectable viral load and symptomatic clinical disease at the end of follow-up compared to recruitment, with the prevalence of subjects with at least one of detectable viral load, symptomatic disease, or immunosuppression being 70.00% (*n* = 287).

- All subjects who were ART-naïve at recruitment commenced ART at some point during follow-up

- The prevalence of LS increased over follow-up to 61.1% (*n* = 253): however, the proportions of subjects defined by body fat alterations alone, metabolic abnormality alone, and by both fat alterations and metabolic abnormality was consistent with those seen at recruitment.

- While the percentage distributions of subjects with specific fat alterations were similar to those seen at recruitment, the percentage distributions of specific metabolic abnormalities were different: at both recruitment and the end of follow-up approximately one-third of subjects with metabolic abnormality were defined by fasting hypertriglyceridemia alone, while the percentage defined by hypercholesterolemia increased from 36.1% to 51.7%, and the percentage defined by mixed abnormality fell from 23.1% to 13.8%.

- The incidence of new LS cases was estimated to be 17.8 per 100 years of person-time (95% CI: 14.1, 21.5), with a higher incidence of body fat alterations compared with metabolic abnormality: arm lipoatrophy, trunk lipohypertrophy, and hypercholesterolemia emergence were seen most frequently.

- Use of PIs at recruitment was a significant and independent risk factor for incident metabolic abnormality, being associated with an approximate 4-fold increase in risk in multivariable models. In contrast, while moderate CDC-defined clinical status was associated with a 2-3 fold increase in risk for incident LS), White ethnicity was associated with an 85% decrease in risk in multivariable Cox models. Immunosuppression over the follow-up period was associated with a significant 60% reduced risk of incident body fat alterations in multivariable modelling.

- Over the follow-up period, 221 subjects had both regression and progression of LS symptoms, most commonly manifesting as progression of metabolic abnormality with accompanying regression of body fat alterations (n = 95).

- There were no significant differences in any subject characteristic (including HIV-related factors and ART regimen) with change in symptom severity of either body fat alterations or metabolic abnormality.

7. Relationship between body fat alterations and metabolic abnormality

7.1 Objectives

This chapter investigates the temporal relationship between body fat alterations and metabolic abnormality, models the relationship between LS and serum concentrations of metabolites, and investigates strategies used in the management of LS symptoms in this cohort.

Specific objectives include:-

- Description of the metabolic profile in subjects with incident body fat alterations over follow-up and investigation as to whether recruitment metabolic abnormality is a risk factor for incident body fat alterations.

- Description of body fat habitus in subjects with incident metabolic abnormality over follow-up and investigation as to whether recruitment body fat alteration is a risk factor for incident metabolic abnormality.

- Modelling longitudinal changes in concentration of cholesterol and fasting triglyceride and their association with LS

- Examination of management of LS with identification of possible indicators used for treatment.

7.2 Specific methods

7.2.1 Description of metabolic profile in subjects with incident body fat alterations

Subjects with incident body fat alterations over follow-up were identified as outlined in the previous chapter. Comparisons of median levels of metabolic outcomes at recruitment and at the end of follow-up in these subjects were made using the Signed-rank test for paired medians. The metabolic outcomes examined include median levels of: fasting triglyceride, total cholesterol, LDL-cholesterol, HDL-cholesterol and non-HDL cholesterol.

7.2.2 Description of body fat alterations in subjects with incident metabolic abnormality

The proportion of subjects with incident metabolic abnormality by (i) severity of lipohypertrophy symptoms, (ii) severity of lipoatrophy symptoms, and (iii) manifestation of body fat alterations, (i.e. lipohypertrophy, lipoatrophy or both) at both recruitment and end of follow-up were compared using Chi squared tests (Chapter 2: Section 2.8.3).

7.2.3 Time-to-event analyses

Time-to-event analyses were conducted to determine whether metabolic abnormality was a risk factor for incident body fat alterations, and vice versa. Two sets of time-to-event analyses, using Cox proportional hazards models, were conducted:-

1. Models with incident body fat alterations as the outcome, and metabolic abnormality included in the explanatory variables
2. Models with incident metabolic abnormality as the outcome, and body fat alterations included in the explanatory variables.

For each set of analyses, several models were fitted:

- Kaplan-Meier estimates investigating the probability of incidence in subjects between groups
- A univariable model with the relevant recruitment LS explanatory variable
- A multivariable model with covariates selected by backward covariate selection as outlined in Chapter 2 (explanatory variables summarized in Table 2-1). All final models retained the relevant recruitment LS explanatory variable, age, duration of ART use at recruitment and a random effect on the intercept for clinical site, regardless of their statistical significance in the model.
- A second multivariable model with covariates selected by backward model selection retaining the relevant time-updated LS explanatory variable, age, duration of ART use

at recruitment and a random effect term on the intercept for clinical site, regardless of their statistical significance in the model

- A multivariable model with the relevant recruitment LS explanatory variable, and ART regimen at recruitment: intuitively adjusted for age and duration of ART at recruitment, and including a random effect on the intercept for clinical site. All models were intuitively adjusted by the same covariates to facilitate comparison between models.

Sensitivity analyses could not be performed on these models because few cases of moderate/severe LS occurred: for example, only 7 cases of moderate/severe fat alterations (from a previous symptom-free state) occurred during follow-up.

7.2.4 Longitudinal analyses

The effect of LS on metabolic outcomes (Box 7-1) was investigated using multilevel modelling as described in Chapter 2.

Box 7-1: Serum concentrations of metabolites (mg/dL) modelled by multilevel approach

Total cholesterol
Low density lipoprotein cholesterol
Non-high density lipoprotein cholesterol
High density lipoprotein cholesterol
Fasting triglyceride

All models were 3-level with subject clustered in clinical site in the random intercept. For each outcome, 4 separate multivariable models examining the influence of LS were investigated:

1. Recruitment variables included in the final model chosen by stepwise covariate-selection (including categories of ART used at recruitment)
2. Time-updated variables included in the final model by stepwise model selection (including time-updated categories of ART use)
3. Model investigating LS and ART regimen at recruitment intuitively adjusted by age at recruitment, sex, ethnicity, BMI at recruitment, maximum CDC-defined clinical status, degree of CDC-defined immunosuppression at recruitment, and detectable viral load at recruitment.
4. Models investigating time-updated LS and ART regimen which was intuitively adjusted as for 3.

The variables included in the fixed part of the final multivariable models are summarized Table 7-1.

Table 7-1: Explanatory variables in multivariable models

		Models investigating factors at recruitment	Models investigating time updated variables
Lipodystrophy syndrome	At recruitment	X	
	Time-updated		X
Ethnicity		X	X
Body mass index	At recruitment	X	X
Detectable viral load	At recruitment	X	
	Time-updated		X
CDC-defined HIV symptoms	Time-updated		X
Maximum CDC-defined HIV symptoms		X	X
CDC-defined HIV immunosuppression	Time-updated		X
Protease inhibitor use	At recruitment	X	
	Time-updated		X
Non-nucleoside reverse transcriptase inhibitor use	At recruitment	X	
	Time-updated		X
Antiretroviral therapy use	At recruitment	X	
	Time-updated		X

All models include age at recruitment, duration of drug use at recruitment, and sex

Confounding variables included in models 3 and 4 above were selected due to their statistical significance in univariable models and in multivariable models 1 and 2. However, it is important to note that the explanatory variable LS is related to the outcome variables of the models as the definition of LS may incorporate hypercholesterolemia, fasting hypertriglyceridemia, or both. The method of stepwise selection of variables was as described in Section 2.8 in Chapter 2.

Log ratio tests were conducted to compare the fit of the 3-level model with either a 2-level (random intercept for subject only) or null model (no random intercept). The normality assumption for 2-level and 3-level residuals was investigated using quantile-quantile (QQ) plots.

7.2.5 Strategies for management of lipodystrophy syndrome

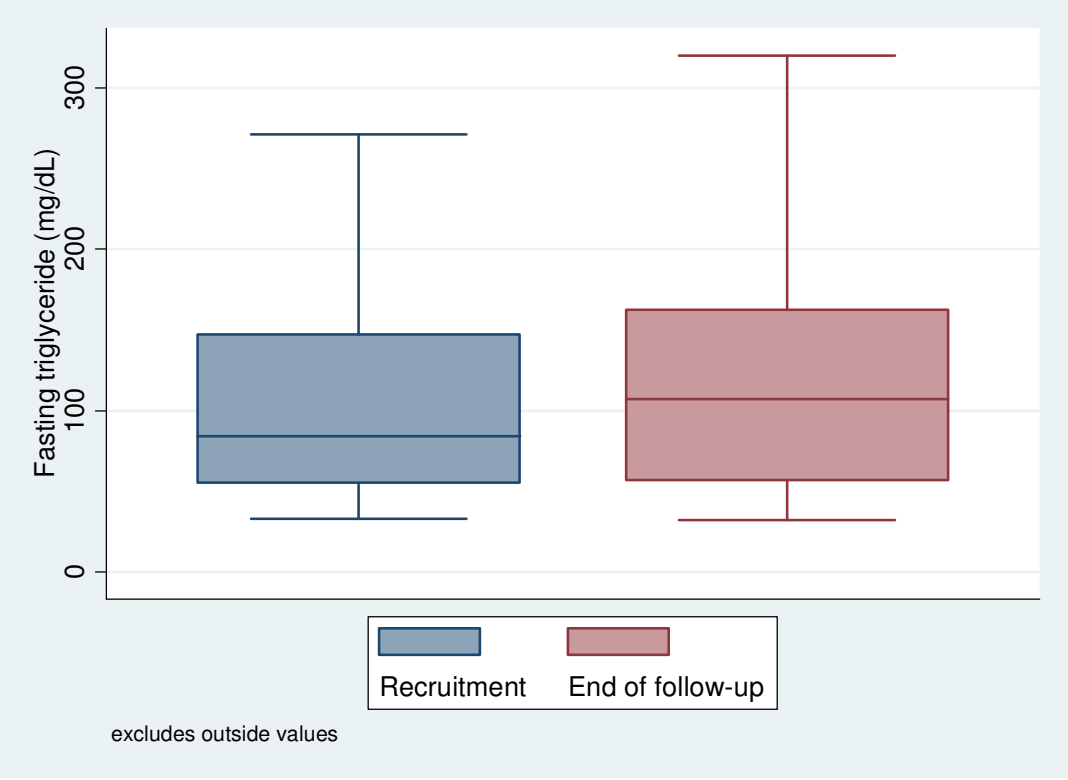
Among prevalent LS cases at recruitment, the proportion receiving pharmacological, dietary or physical intervention at any time during follow-up was calculated. To explore the most common management strategies, the distribution of combinations of these interventions among subjects with LS was estimated at both recruitment and the end of follow-up. Comparison of the proportion of subjects with each intervention and specific symptoms (LS, body fat alterations, or metabolic abnormality) at recruitment and then the end of follow-up was made using Chi-squared tests. Specific pharmaceutical interventions are described in relation to initial and final symptoms of subjects.

7.3 Results

7.3.1 Metabolic profile of subjects with incident body fat alterations

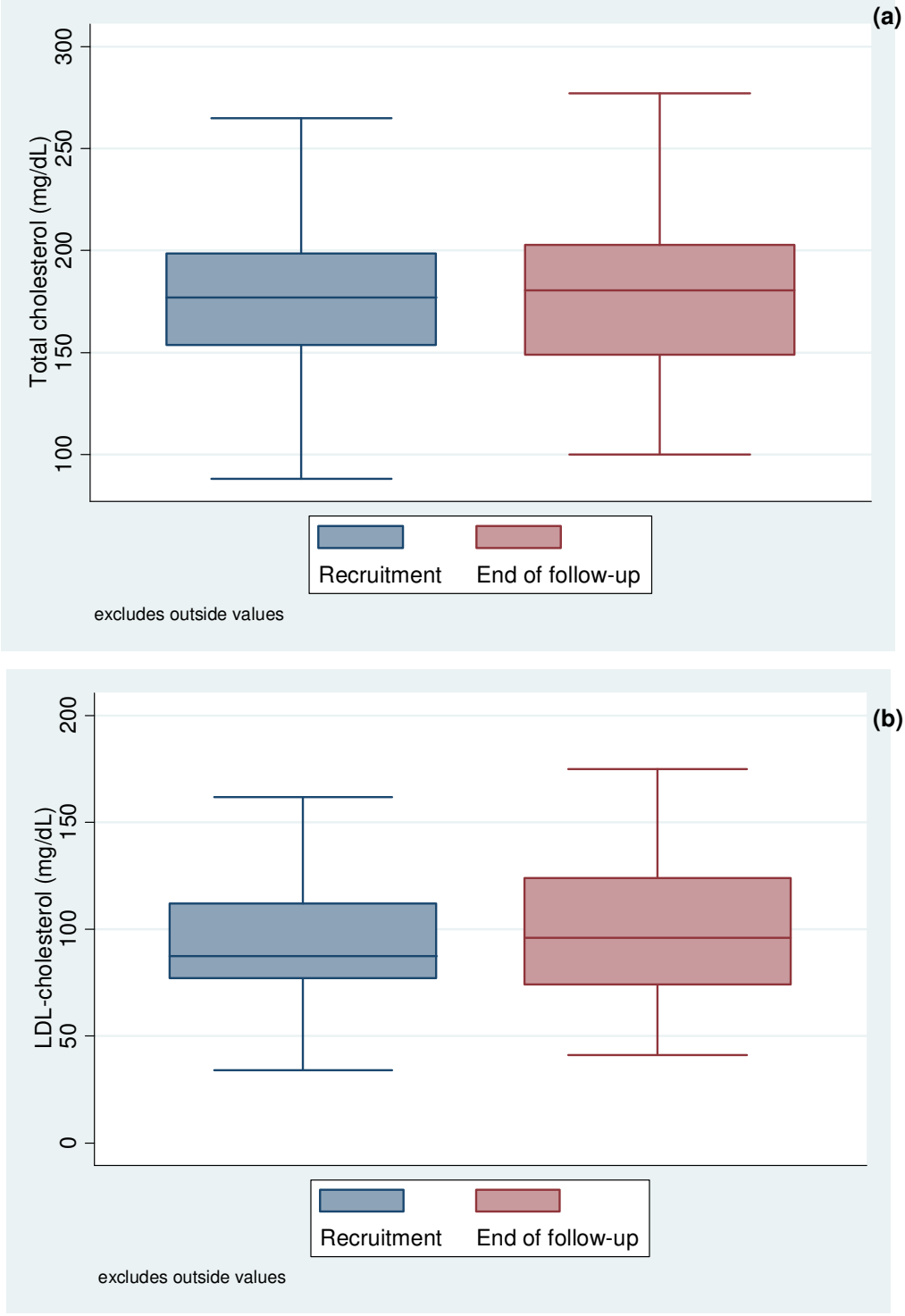
Among subjects with incident body fat alterations, median total fasting triglyceride was non-significantly (p = 0.666, n = 60) higher at the end of follow-up compared to recruitment (Figure 7-1); although recruitment levels were significantly (p = 0.006) higher in male adolescents aged 12-18 years (179.0mg/dL, IQR: 83.0, 253.0, n = 12) compared to the end of follow-up (107.0mg/dL, IQR: 58.0, 133.0, n = 16), significant differences were not seen for any other age/sex group (Appendix G: Table G-1). Total cholesterol (p = 0.609, n = 64), LDL-cholesterol (p = 0.229, n = 63: Figure 7-2), and non-HDL cholesterol (p = 0.999, n = 60, Figure 7-3b) were non-significantly higher at the end of follow-up compared to recruitment, whilst median HDL-cholesterol was non-significantly lower (p = 0.435, n = 63, Figure 7-3a).

Figure 7-1: Comparison of median levels of fasting triglyceride at recruitment and the end of follow-up in subjects with incident body fat alterations



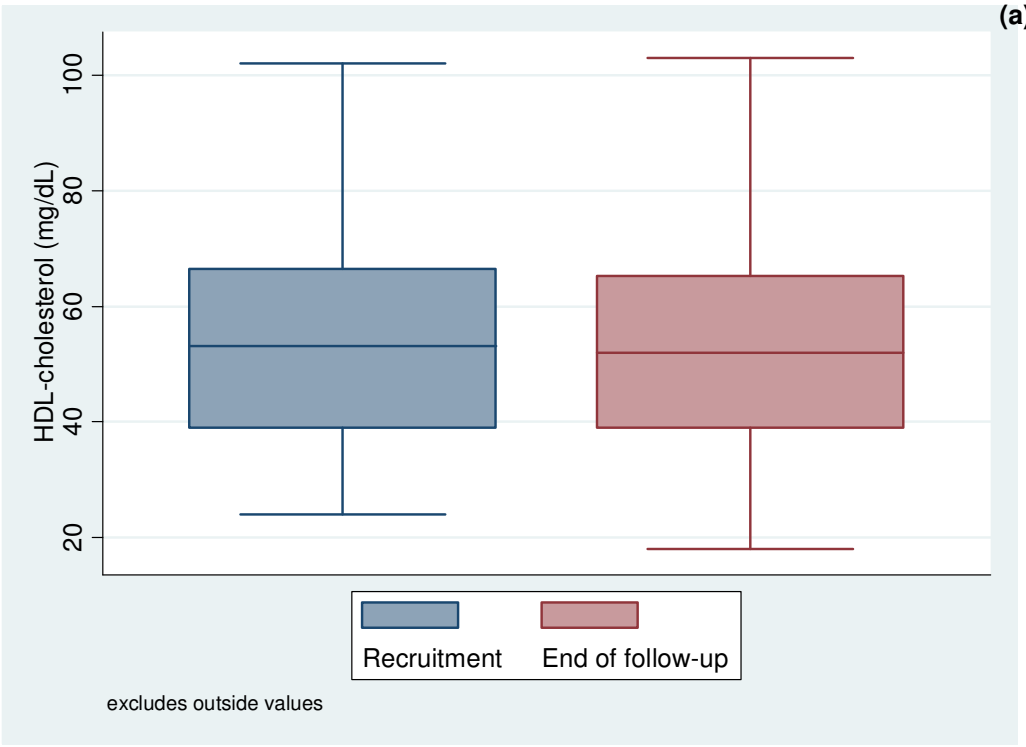
Median and IQR: recruitment fasting triglyceride; 84.0mg/dL (55.0, 147.9, n =50), follow-up fasting triglyceride; 107mg/dL (56.3, 162.6, n = 60); signed-rank test for paired medians; p = 0.666

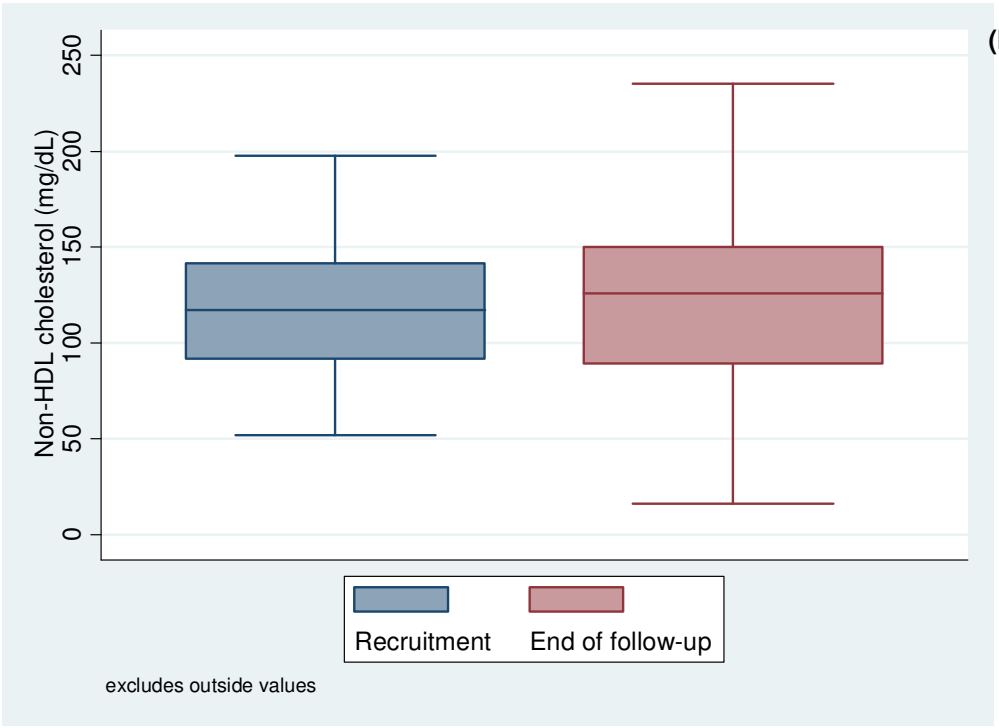
Figure 7-2: Comparison of median levels of (a) total cholesterol, and (b) LDL-cholesterol, at recruitment and at the end of follow-up in subjects with incident body fat alterations



Median and IQR: recruitment total cholesterol; 177.0 (153.3, 198.8, *n* =64), follow-up total cholesterol; 180mg/dL (147.3, 203.1, *n* =64); signed-rank test for paired medians; *p* = 0.609. Median and IQR: recruitment LDL-cholesterol; 87.4 (76.8, 112.5, *n* =58), follow-up LDL-cholesterol; 96.0mg/dL (74.0, 124.0, *n* =63); signed-rank test for paired medians; *p* = 0.229.

Figure 7-3: Comparison of median levels of (a) HDL-cholesterol, and (b) non- HDL-cholesterol, at recruitment and at the end of follow-up in subjects with incident body fat alterations



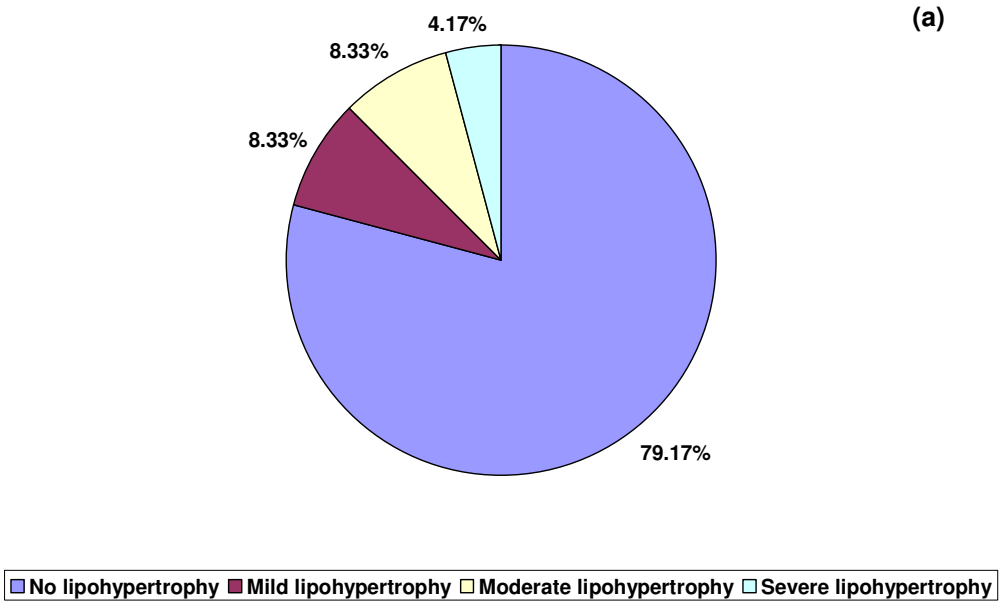


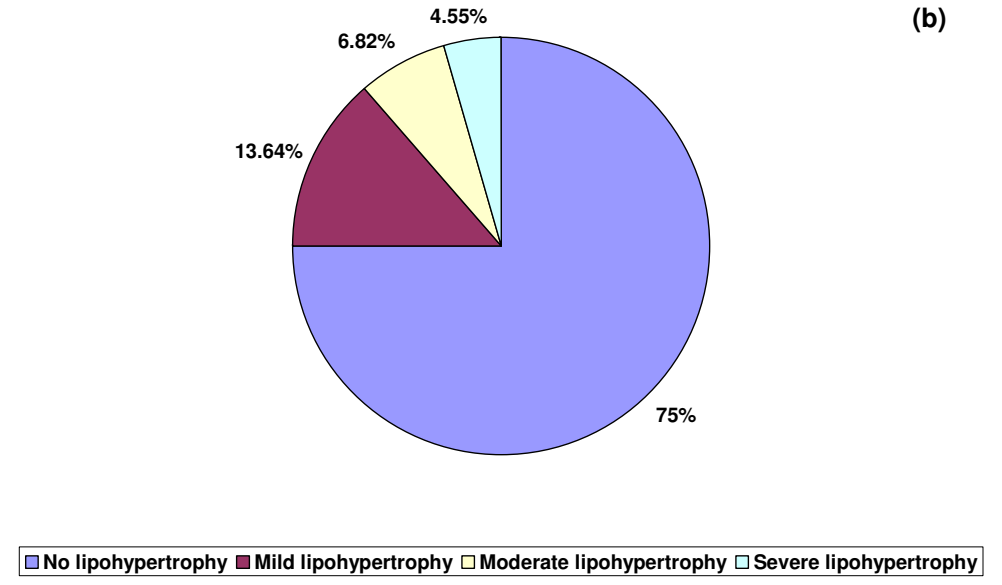
Median and interquartile range HDL-cholesterol; 53.2 (95% CI: 39.0, 66.8, *n* =60), follow-up HDL-cholesterol; 52.0 (39.0, 65.3, *n* =63); signed-rank test for paired medians; *p* = 0.435. Median and IQR: recruitment non-HDL cholesterol; 117.0mg/dL (91.3, 142.3, *n* =60), non-HDL cholesterol; 126mg/dL (89.0, 150.0, *n* =63): signed-rank test for paired medians; *p* = 1.000

7.3.2 Body fat alterations in subjects with incident metabolic abnormality

Among the 48 children and adolescents with incident metabolic abnormality, there were significant (p<0.001) increases in the proportion with any body fat alterations (Figure 7-4), lipohypertrophy (Figure 7-5) and lipoatrophy (Figure 7-6) between recruitment and the end of follow-up.

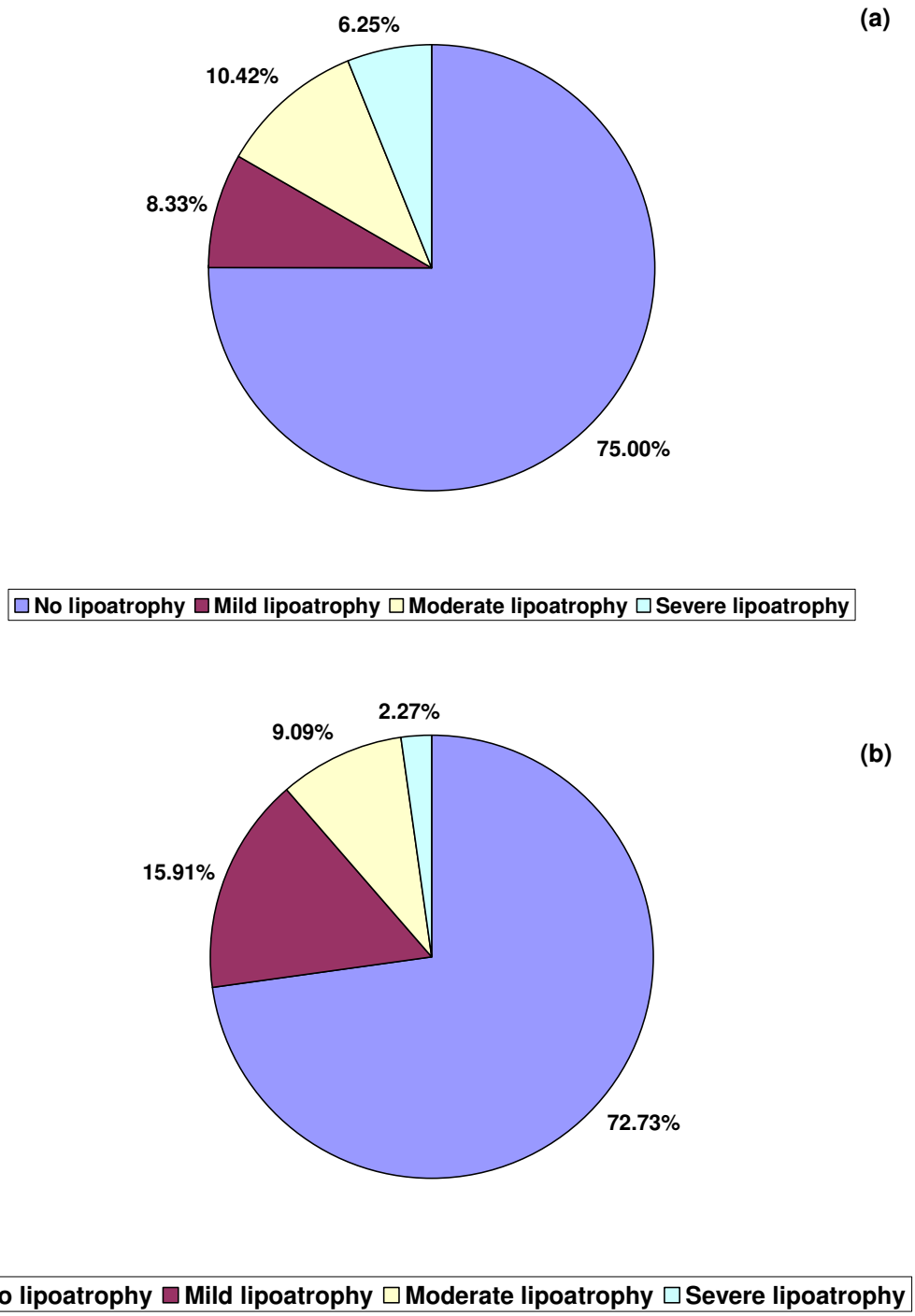
Figure 7-4: **Comparison of lipohypertrophy in subjects with incident metabolic abnormality (n = 48) at (a) recruitment, and (b) end of follow-up**





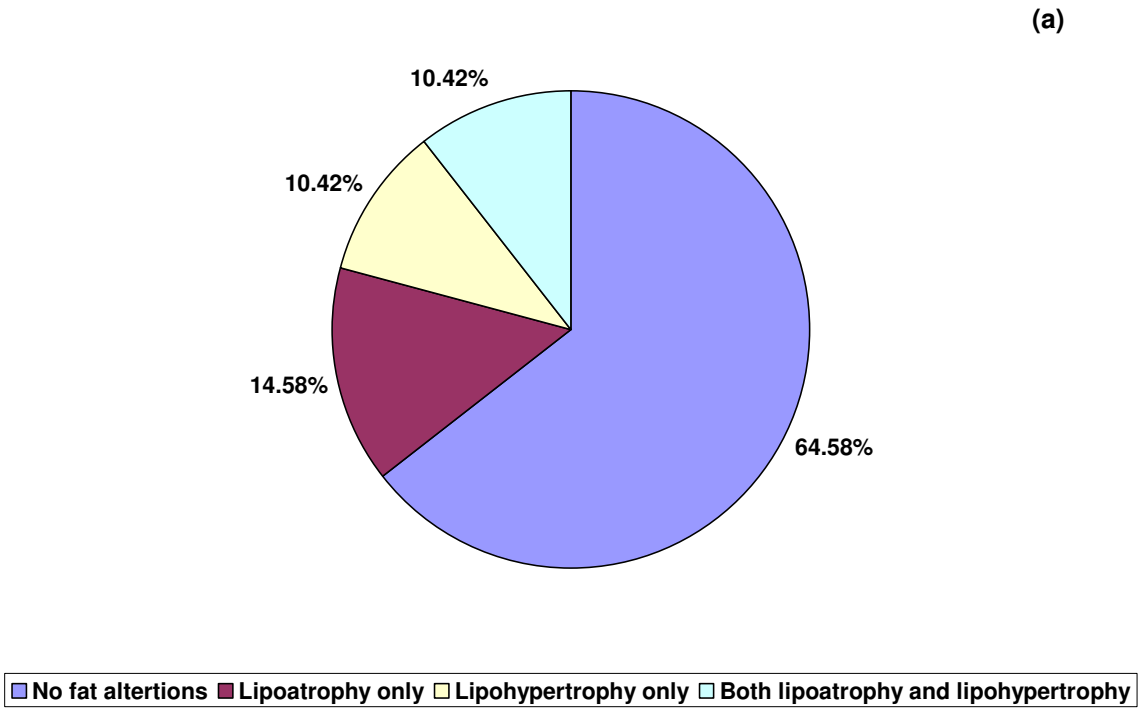
χ^2 test : p < 0.001

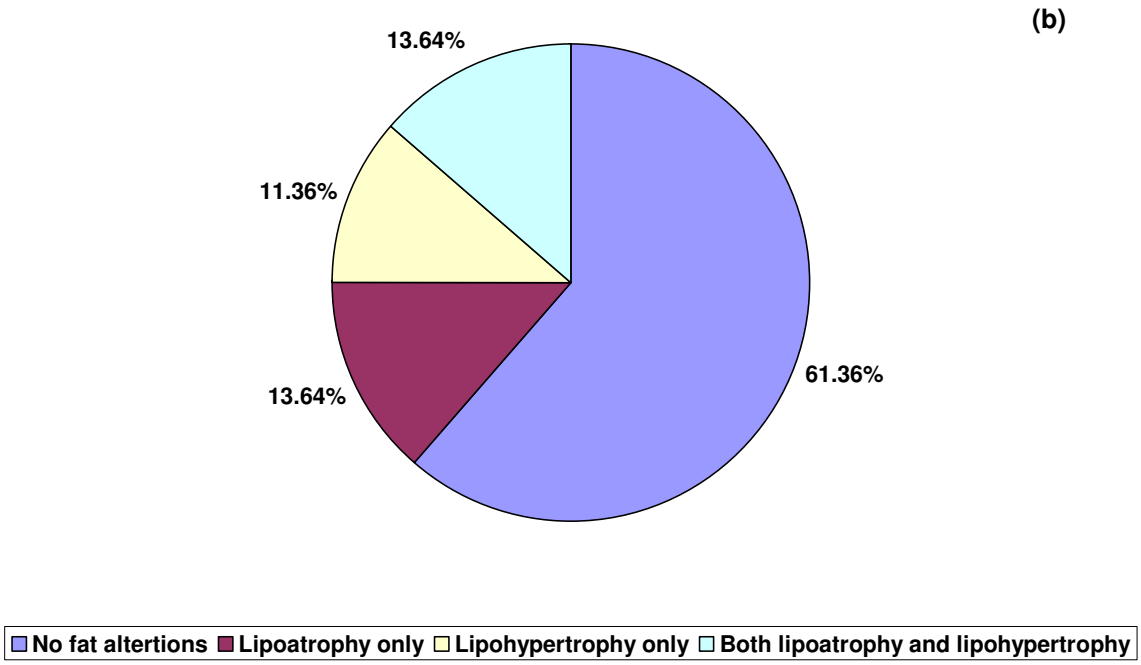
Figure 7-5: **Comparison of lipoatrophy in subjects with incident metabolic abnormality (*n* = 48) at (a) recruitment, and (b) end of follow-up**



χ^2 **test : $p < 0.001$**

Figure 7-6: Body fat alterations in subjects with incident metabolic abnormality (*n* = 48) at (a) recruitment, and (b) end of follow-up





χ^2 test : $p < 0.001$

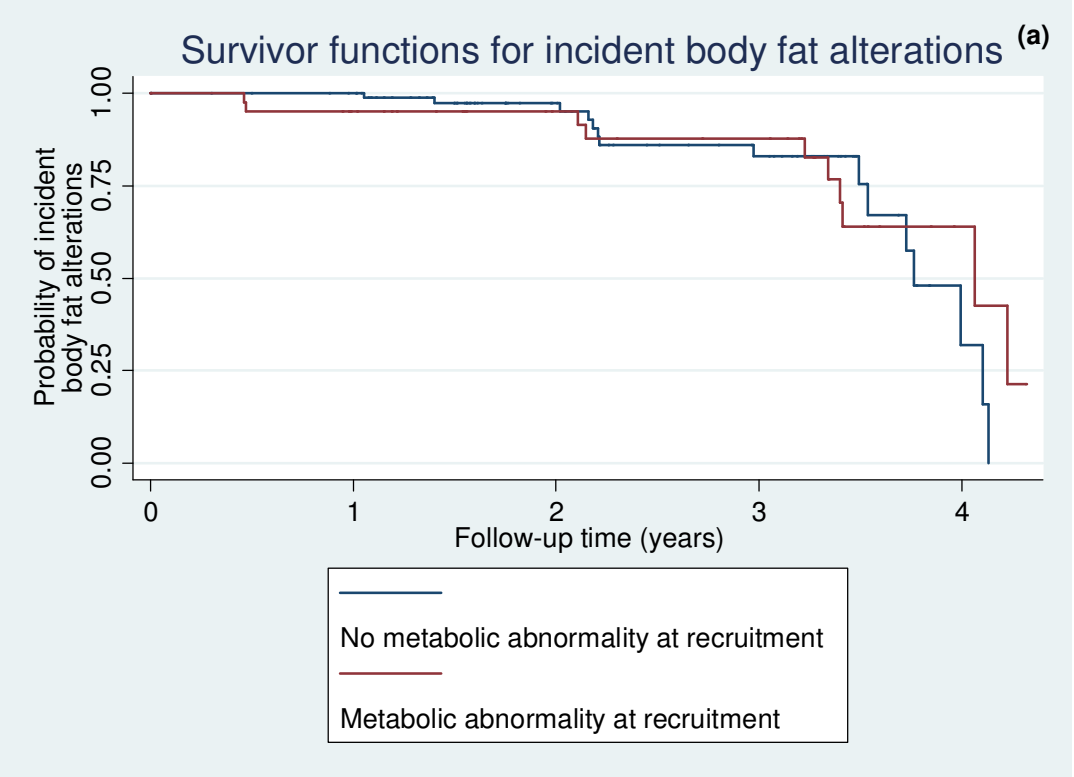
7.3.3 Relationship between metabolic abnormality and body fat alterations

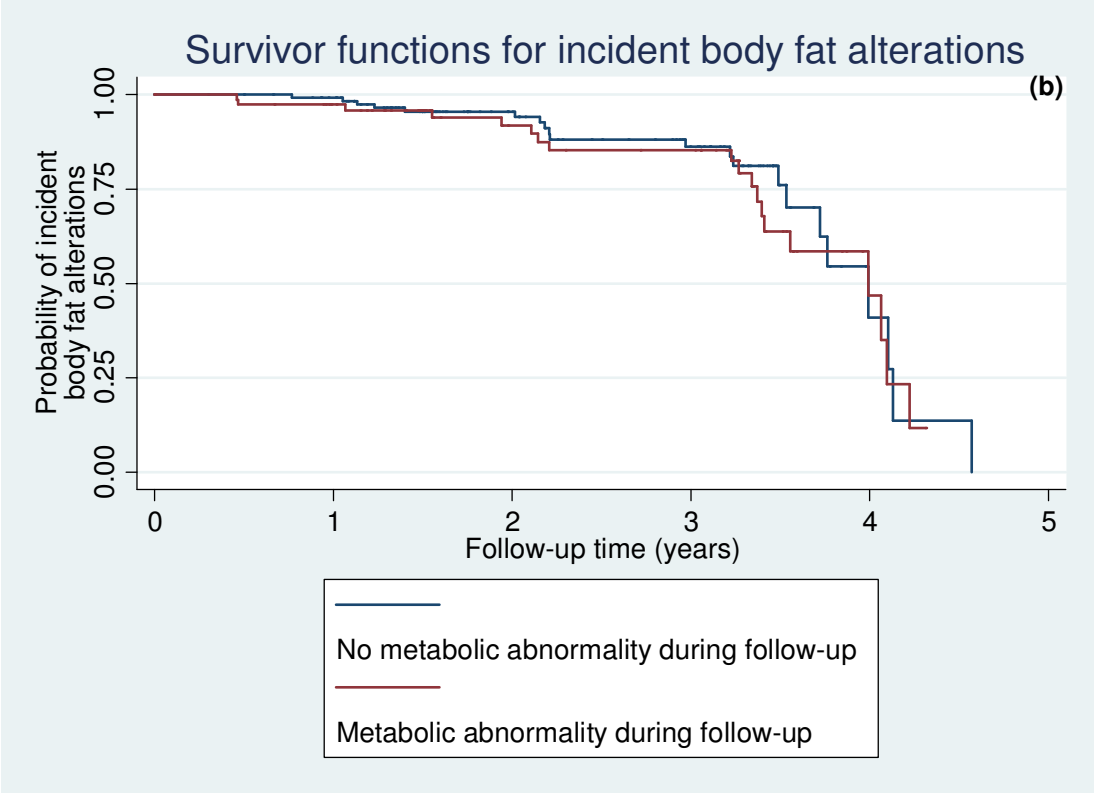
During the first 2 years of follow-up, the probability of incident body fat alterations was greater amongst subjects with no metabolic abnormality at recruitment (Figure 7-7a). However, by the end of 4 years, the probability of developing new body fat alterations was approximately 60% among subjects with metabolic abnormality at recruitment compared to approximately 45% in subjects without. The probability of incident body fat alterations among subjects with any metabolic abnormality over follow-up and among subjects without was similar over the whole study period: respective probabilities at 4 years were approximately 20% and 10% (Figure 7-7b).

In contrast, subjects with body fat alterations at recruitment had a greater probability of incident metabolic abnormality for most of the follow-up period: the estimated probability was 90% in subjects with recruitment body fat alterations compared to 80% in subjects without at 3 years of follow-up (Figure 7-8a). Similarly, the probability of incident metabolic abnormality was greater in subjects who had body fat alterations over the follow-up period compared to those who did not, for the majority of the follow-up period: percentages at 3 years were similar to those seen with recruitment body fat abnormality (Figure 7-8b).

In all cases, there was no significant difference in probability function for incidence between groups ($p \geq 0.365$)

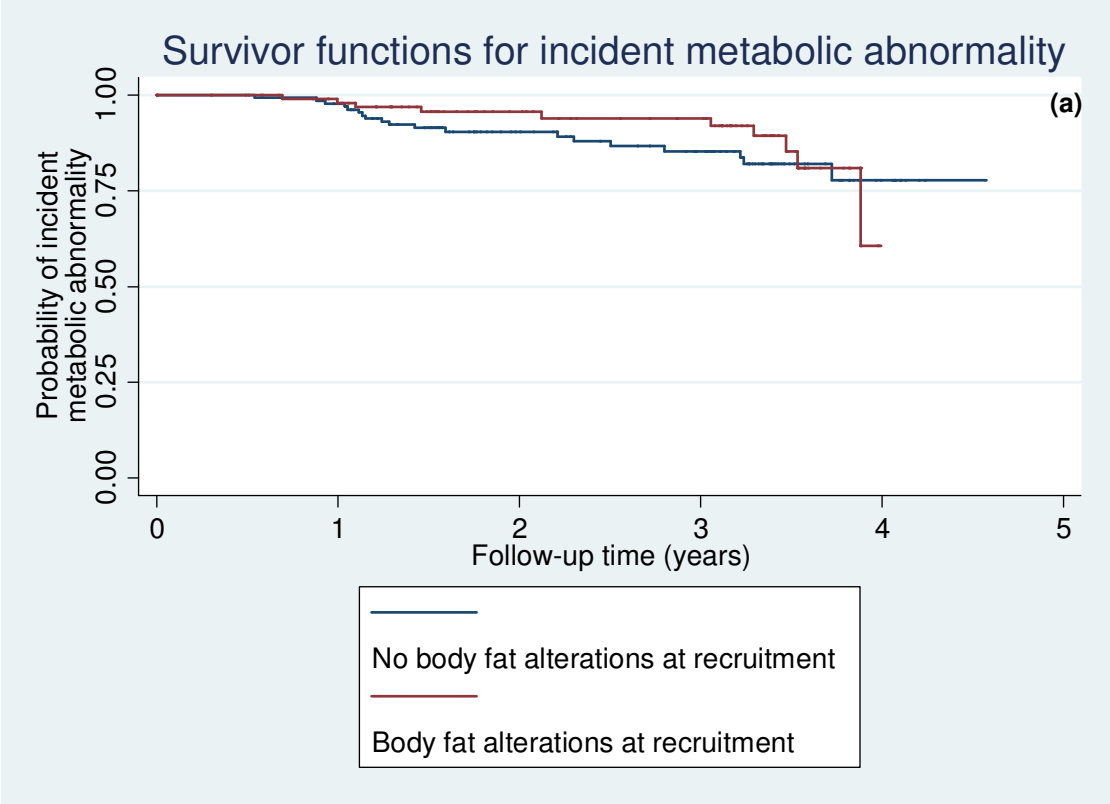
Figure 7-7: Kaplan-Meier estimates for incident body fat alterations by (a) recruitment metabolic abnormality, and (b) time-updated metabolic abnormality

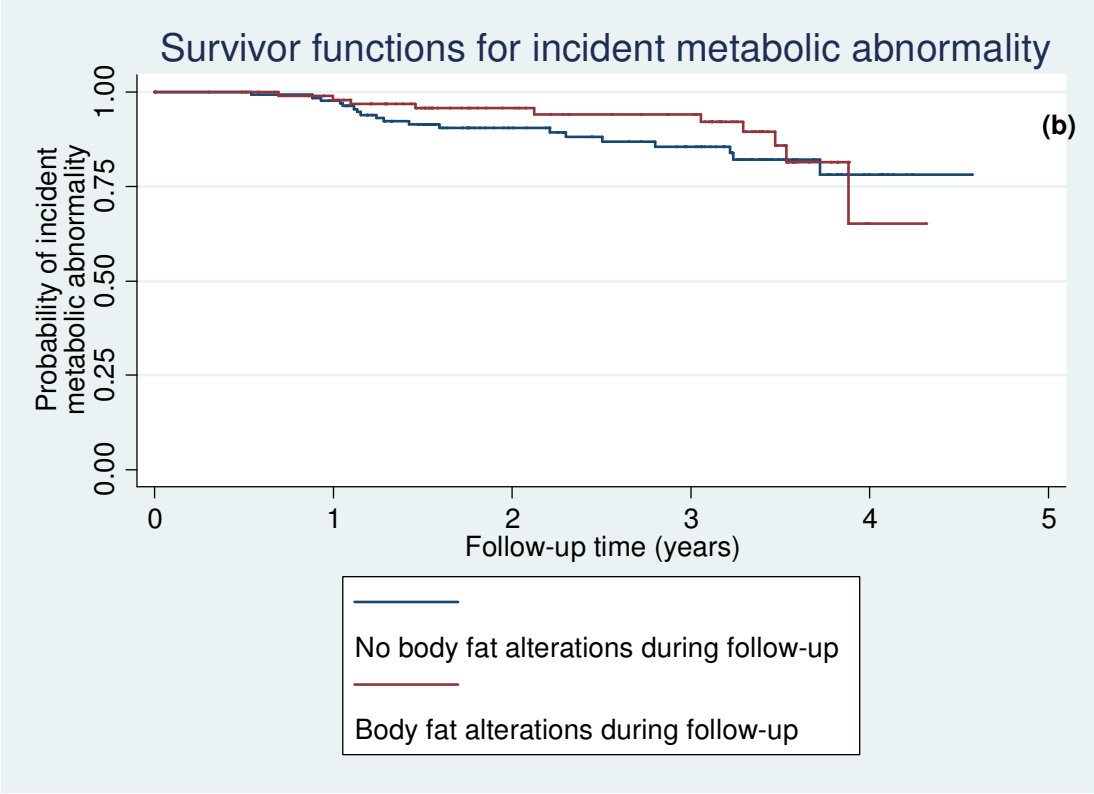




Log rank test for equality of survival functions: (a) χ^2 statistic = 0.82 p = 0.365 (b) χ^2 statistic = 0.01 p = 0.919

Figure 7-8: Kaplan-Meier estimates for incident metabolic abnormality by (a) recruitment body fat alterations, and (b) time-updated body fat alterations





Log rank test for equality of survival functions: (a) χ^2 statistic = 0.42 p = 0.516 (b) χ^2 statistic = 0.45 p = 0.500

In univariable Cox proportional hazards regression models, neither metabolic abnormality at recruitment nor metabolic abnormality occurring during follow-up was significantly associated with incident body fat alterations (Table 7-2).

Table 7-2: Univariable models investigating metabolic abnormality as a risk factor for body fat alterations

	n	Hazard ratio	95% confidence interval	*p*-value
Recruitment metabolic abnormality	200	0.88	(0.38, 2.02)	0.757
Time-updated metabolic abnormality	237	1.03	(0.58, 1.84)	0.919

In comparison to the univariate analyses metabolic abnormality was a significant risk factor in multivariable models adjusted by age, duration of ART use clinical condition, immunosuppression and metabolic abnormality. The presence of metabolic abnormality at recruitment was associated with a significant increased risk of incident body fat alterations (AHR: 5.37, 95% CI: 1.07, 26.90) in multivariable modelling with duration of ART of more than 10 years the only other significant factor (Table 7-3). Despite this, in the final multivariable investigating body fat alterations with time-updated covariates, metabolic abnormality did not reach statistical significance (Table 7-3): the occurrence of metabolic abnormality during follow-up was associated with a non-significant ($p = 0.775$) increase in risk.

Further models investigating the association of metabolic abnormality and specific ART-regimen with incident body fat alterations were explored: both recruitment and time-updated metabolic abnormality was associated with a non-significant ($p \geq 0.204$) increased risk of incident body fat alterations (Table 7-4).

Table 7-3: **Multivariable model for risk of incident body fat alterations with recruitment/time-updated metabolic abnormality as an explanatory variable**

		Model with recruitment metabolic abnormality* (*n* = 113)			Model with time-updated metabolic abnormality** (*n* = 186)		
		Adjusted hazard ratio	95% confidence interval	*p*-value	Adjusted hazard ratio	95% confidence interval	*p*-value
Age at recruitment	**<12 years**	1			1		
	12-18 years	0.63	(0.16, 2.50)	0.511	1.00	(0.47, 2.15)	0.998
Duration of ART use at recruitment	**<2.5 years**	1			1		
	2.5-4.9 years	1.57	(0.23, 10.55)	0.644	0.89	(0.27, 2.91)	0.848
	5-7.49 years	2.69	(0.19, 38.54)	0.465	1.42	(0.41, 4.93)	0.586
	7.5-9.99 years	2.58	(0.42, 15.68)	0.303	1.89	(0.72, 4.95)	0.195
	≥10 years	6.25	(1.12, 34.86)	0.037	1.42	(0.48, 4.26)	0.525
CDC-defined clinical condition	**N/A**	1					
	B	0.12	(0.01, 1.55)	0.106			
	C	0.41	(0.06, 2.74)	0.355			
CDC-defined immunosuppression	**None**	1					
	Moderate	0.25	(0.06, 1.03)	0.056			
	Severe	1.26	(0.18, 8.70)	0.813			
Metabolic abnormality	**Absent**	1			1		
	Present	5.37	(1.07, 26.90)	0.041	1.12	(0.52, 2.40)	0.775
Protease inhibitor	**No use**	1					
	Use	0.28***	(0.07, 1.19)	0.085			

*** Therneau-Grambsch global test of non-proportionality: *p* = 0.181, ** Therneau-Grambsch global test of non-proportionality: *p* = 0.905 ***Use at recruitment**

Table 7-4: Multivariable model for risk of incident body fat alterations with recruitment/time-updated metabolic abnormality and treatment regimen as explanatory variables

		Model with recruitment metabolic abnormality* (*n* = 187)			Model with time-updated metabolic abnormality** (*n* = 159)		
		Adjusted hazard ratio	95% confidence interval	*p*-value	Adjusted hazard ratio	95% confidence interval	*p*-value
Age at recruitment	**<12 years**	1			1		
	12-18 years	2.56	(0.70, 9.31)	0.154	2.63	(1.06, 6.53)	0.037
Duration of ART use at recruitment	**<2.5 years**	1			1		
	2.5-4.9 years	0.13	(0.01, 1.11)	0.062	0.61	(0.19, 1.95)	0.406
	5-7.49 years	0.81	(0.15, 4.42)	0.804	1.09	(0.31, 3.76)	0.894
	7.5-9.99 years	1.08	(0.32, 3.64)	0.900	1.32	(0.47, 3.68)	0.598
	≥10 years	0.22	(0.04, 1.28)	0.091	0.26	(0.05, 1.37)	0.112
Metabolic abnormality	**Absent**	1			1		
	Present	1.78	(0.54, 5.87)	0.347	2.00	(0.83, 4.80)	0.120
ART regimen at recruitment	**PI-based HAART**	1			1		
	NRTI mono-therapy	0.35	(0.07, 1.68)	0.189	0.48	(0.15, 1.51)	0.212
	NNRTI-based HAART	2.17	(0.66, 7.18)	0.204	1.02	(0.40, 2.62)	0.967
	Triple class therapy	-	-	-	-	-	-

*** Therneau-Grambsch global test of non-proportionality: $p < 0.001$, ** Therneau-Grambsch global test of non-proportionality: $p = 0.838$**

7.3.4 Body fat alterations as a risk factor for incident metabolic abnormality

Children with recruitment body fat alterations and those with any body fat alterations occurring during follow-up had a non-significant reduced risk of incident metabolic abnormality (Table 7-5).

Table 7-5: Univariable models for incident metabolic abnormality using body fat alterations as the explanatory variable

	n	Hazard ratio	95% confidence interval	*p*-value
Recruitment body fat alterations	292	0.79	(0.40, 1.59)	0.516
Time-updated body fat alterations	298	0.79	(0.39, 1.58)	0.501

The association of body fat alterations with incident metabolic abnormality was further explored in multivariable models: neither body fat alterations at recruitment nor time-updated alterations were significant ($p \geq 0.351$) risk factors in either of the final models (Table 7-6).

Finally, models investigating the association between both body fat alterations and specific ART regimen with incident metabolic abnormality were investigated. Although body fat redistribution at recruitment and time-updated alterations were associated with reduced risk of incident body fat alterations in intuitively adjusted multivariable models including ART regimen at recruitment, these associations were also not statistically significant (Table 7-6).

In common with models presented in Chapter 6, PI was associated with significant increased risk of incident metabolic abnormality: recruitment PI (AHR: 3.86, 95% CI: 1.29, 11.55), and time-updated PI (AHR: 4.22, 95% CI: 1.22, 14.59), as illustrated in Table 7-7 Furthermore, NNRTI-based HAART was associated with a significant decreased risk of incident metabolic abnormality compared to PI-based HAART (Table 7-7).

Table 7-6: Multivariable model for risk of incident metabolic abnormality with recruitment/time-updated body fat alterations as an explanatory variable

		Model with recruitment metabolic abnormality* (*n* = 187)			Model with time-updated metabolic abnormality** (*n* = 178)		
		Adjusted hazard ratio	95% confidence interval	*p*-value	Adjusted hazard ratio	95% confidence interval	*p*-value
Age at recruitment	**<12 years**	1			1		
	12-18 years	1.40	(0.55, 3.57)	0.482	1.19	(0.44, 3.19)	0.730
Duration of ART use at recruitment	**<2.5 years**	1			11		
	2.5-4.9 years	0.72	(0.21, 2.43)	0.592	0.47	(0.12, 1.83)	0.276
	5-7.49 years	0.52	(0.14, 1.99)	0.338	0.55	(0.15, 2.10)	0.385
	7.5-9.99 years	0.57	(0.17, 1.95)	0.370	0.75	(0.21, 2.62)	0.648
	≥10 years	0.73	(0.20, 2.69)	0.640	0.57	(0.14, 2.26)	0.420
Ethnicity	**Black**				1		
	White				0.33	(0.11, 0.98)	0.046
	Other				0.56	(0.06, 4.95)	0.599
Body fat alterations	**Absent**	1			1		
	Present	1.01	(0.42, 2.40)	0.986	4.22	(1.22, 14.59)	0.351
Protease inhibitor	**Absent**	1			1		
	Present	3.86***	(1.29, 11.55)	0.016	4.22^	(1.22, 14.59)	0.023

*** Therneau-Grambsch global test of non-proportionality: *p* = 0.583, ** Therneau-Grambsch global test of non-proportionality: *p* = 0.727. ***Use at recruitment. ^Use during follow-up**

Table 7-7: Multivariable model for risk of incident metabolic abnormality with recruitment/time-updated body fat alterations and treatment regimen as explanatory variables

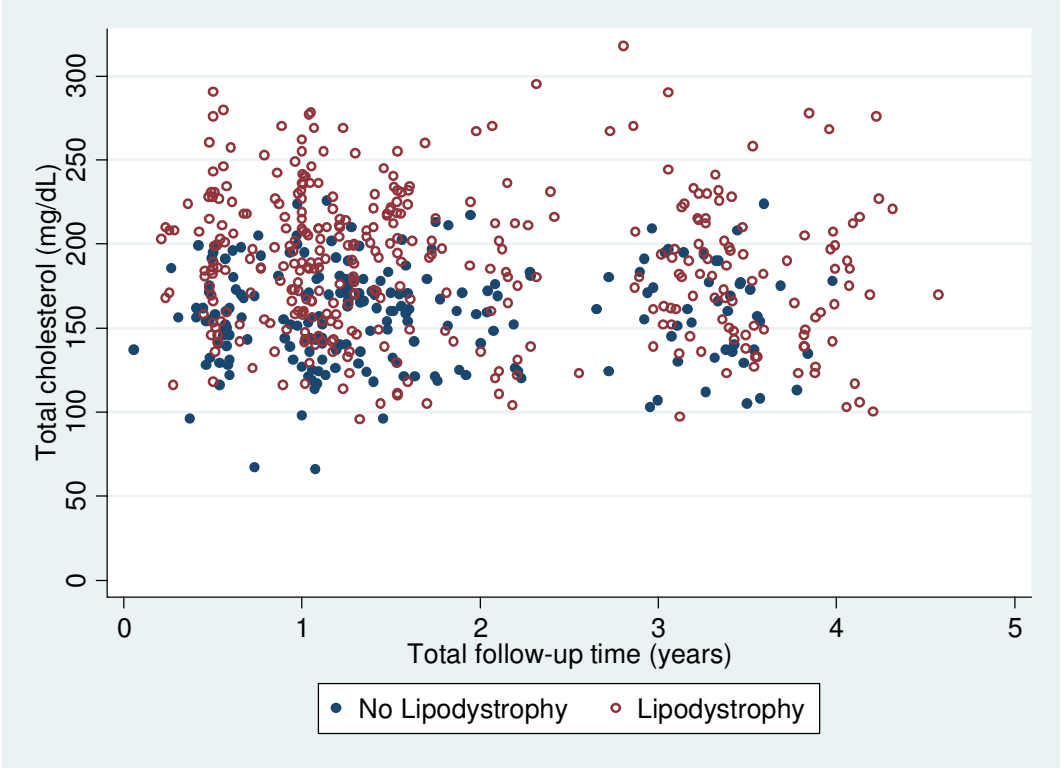
		Model with recruitment body fat alterations* (*n* = 206)			Model with time-updated body fat alterations** (*n* = 189)		
		Adjusted hazard ratio	95% confidence interval	*p*-value	Adjusted hazard ratio	95% confidence interval	*p*-value
Age at recruitment	**<12 years**	1			1		
	12-18 years	1.40	(0.55, 3.59)	0.483	1.35	(0.52, 3.48)	0.533
Duration of ART use at recruitment	**<2.5 years**	1			1		
	2.5-4.9 years	0.70	(0.21, 2.40)	0.574	0.77	(0.22, 2.66)	0.680
	5-7.49 years	0.52	(0.14, 1.99)	0.341	0.57	(0.15, 2.18)	0.408
	7.5-9.99 years	0.54	(0.16, 1.87)	0.330	0.63	(0.18, 2.16)	0.465
	≥10 years	0.69	(0.18, 2.56)	0.574	0.84	(0.23, 3.07)	0.794
Body fat alterations	**Absent**	1			1		
	Present	0.95	(0.39, 2.31)	0.913	0.90	(0.37, 2.22)	0.821
ART regimen at recruitment	**PI-based HAART**	1			1		
	NRTI mono-therapy	0.25	(0.03, 2.03)	0.196	0.26	(0.03, 2.06)	0.201
	NNRTI-based HAART	0.28	(0.08, 0.99)	0.047	0.28	(0.08, 0.96)	0.043
	Triple class therapy	1.99	(0.41, 9.56)	0.390	1.26	(0.26, 6.04)	0.770

*** Therneau-Grambsch global test of non-proportionality: *p* = 0.391, ** Therneau-Grambsch global test of non-proportionality: *p* = 0.200**

7.3.5 Longitudinal modelling of serum cholesterol and fasting triglyceride

Serum levels of total cholesterol were more varied over follow-up among subjects with LS compared to those without, however individual measures appeared to be lower in this second group (Figure 7-9).

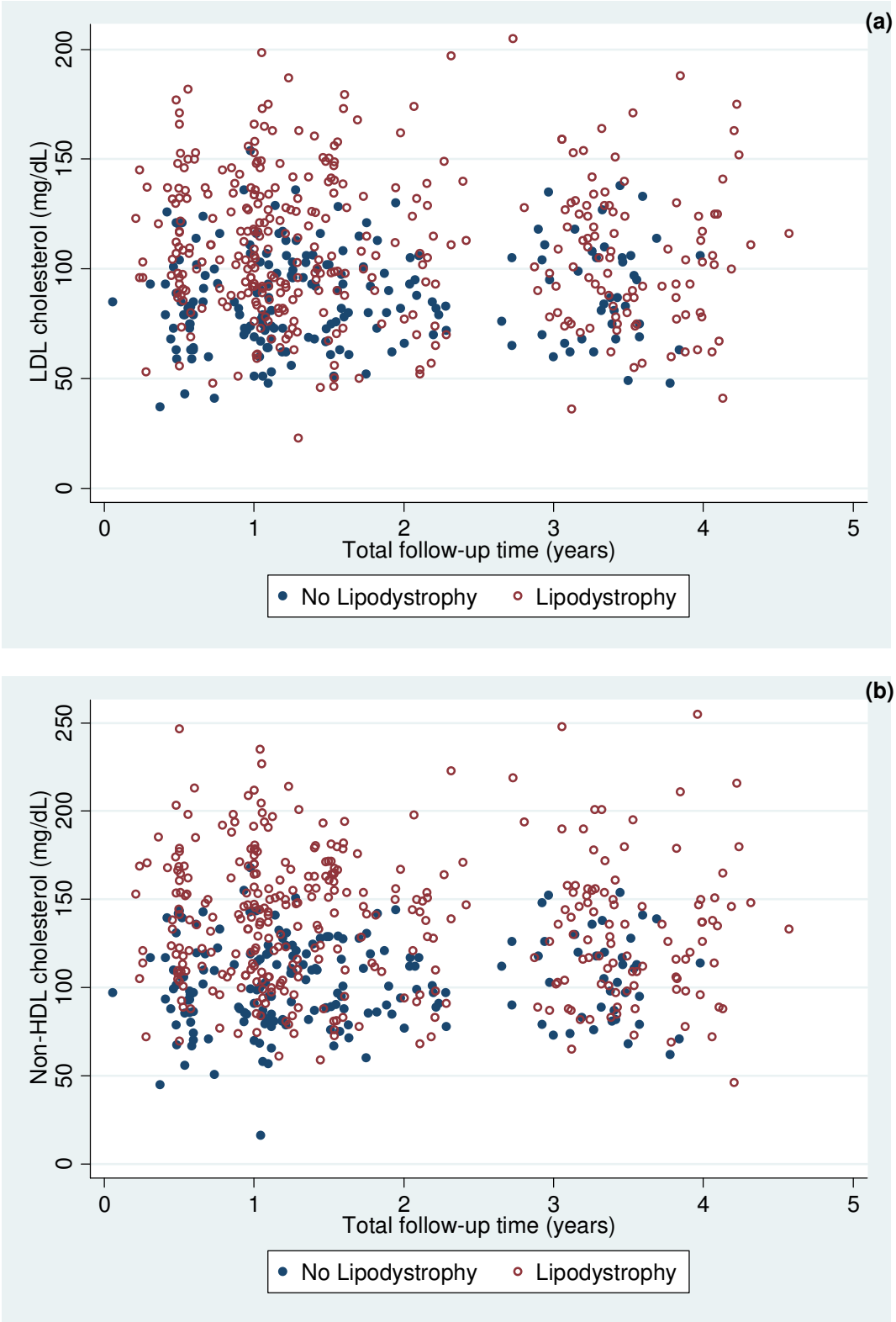
Figure 7-9: Serum measures of total cholesterol over follow-up



Total-cholesterol: number of observations = 537 and *n* = 302

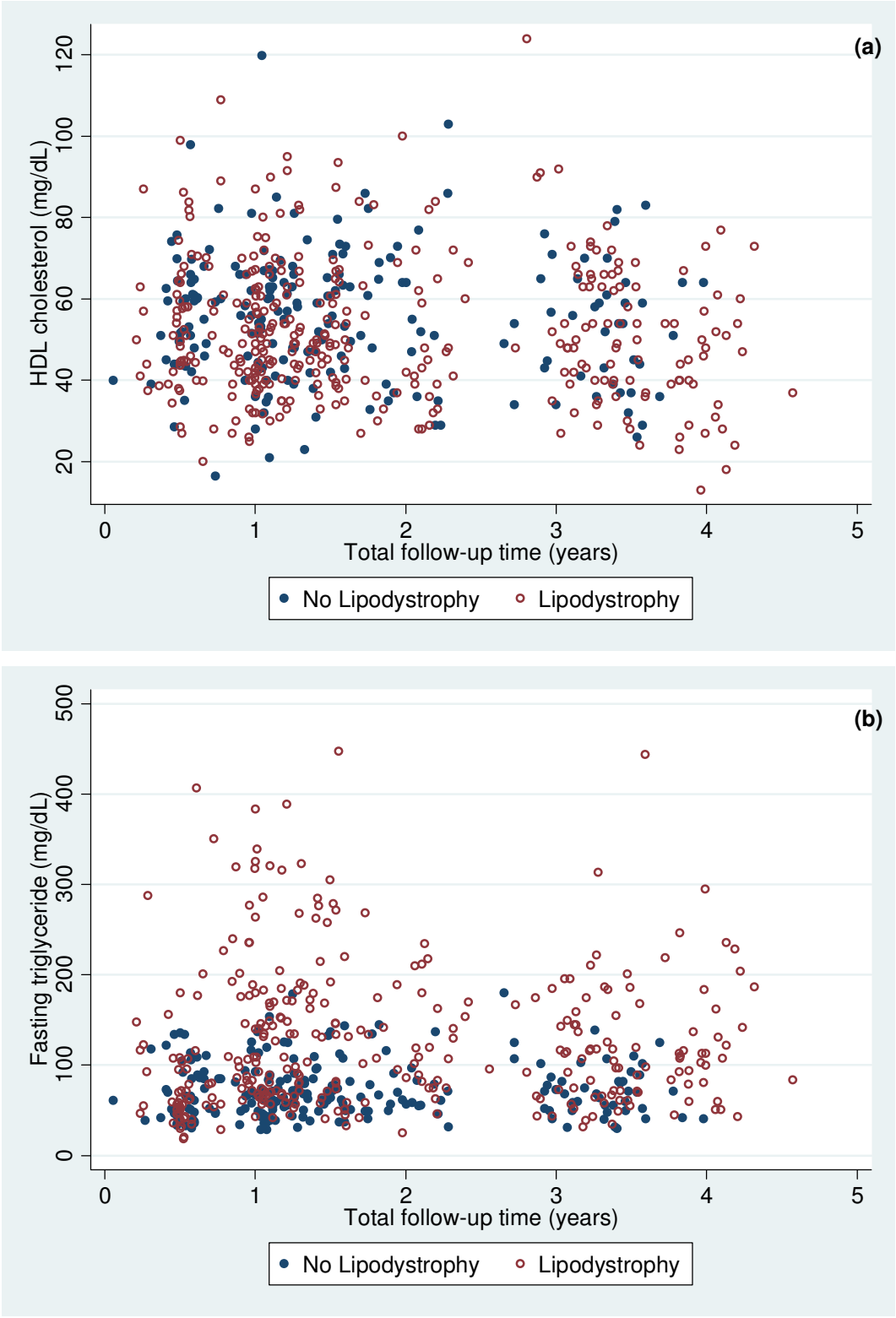
Serum concentrations of LDL-cholesterol and non-HDL cholesterol appeared to be higher in subjects with LS compared to concentrations in subjects without LS over the follow-up period (Figure 7-10). In contrast, similar levels of HDL-cholesterol were seen in subjects with LS compared to those without LS (Figure 7-11a). However, subjects with LS had higher serum concentrations of fasting triglyceride over follow-up compared to those without LS (Figure 7-11b): the spread of values in subjects with LS was greater than the spread seen without LS.

Figure 7-10: Serum measures of (a) LDL-cholesterol and (b) non-HDL cholesterol over follow-up



LDL-cholesterol: number of observations = 548 and *n* =294, and non-HDL cholesterol: number of observations = 546 and *n* = 292

Figure 7-11: Serum measures of (a) HDL-cholesterol and (b) fasting triglyceride over follow-up



HDL-cholesterol: number of observations = 530 and *n* = 292, and fasting triglyceride: number of observations = 537 and *n* = 297

Univariable models

In univariable analyses, the presence of LS at recruitment was associated with significant ($p < 0.001$) 18–42mg/dL increases in all outcomes except HDL-cholesterol as illustrated in (Appendix G: Table G-3). Significant increases of similar magnitude were seen with time-updated LS. Non-significant decreases in HDL-cholesterol were seen with both recruitment and time-updated LS.

Increased age (12-18 years compared to <12 years) was associated with significant reduced serum concentrations (5-18mg/dL) of total, LDL, and non-HDL cholesterol. While White ethnicity was associated ($p \leq 0.042$) with increases in non-HDL-cholesterol and fasting triglyceride: it was also associated ($p < 0.001$) with decreased HDL-cholesterol. Female sex was significantly associated with increased in HDL-cholesterol and decreased fasting triglyceride.

Both recruitment and time-updated detectable viral load was significantly associated with decreases in all metabolic outcomes except fasting triglyceride: with reference to time-updated viral load, significant decreases were seen in all outcomes except HDL-cholesterol. Moderate immunosuppression at recruitment or any immunosuppression over follow-up was significantly associated with small (<20mg/dL) decreases in total, LDL, and HDL-cholesterol. Maximum CDC-defined clinical status B was significantly associated with increased fasting triglyceride, while status C was associated with significant increased LDL-cholesterol, non-HDL cholesterol, and fasting triglyceride.

Duration of ART use at recruitment was significantly associated with changes in all outcomes over follow-up. The greatest decreases were seen in total, LDL, and non-HDL-cholesterol with >10 years of use: however, decreases in fasting triglyceride of a similar magnitude were seen with 2.5-4.9 years of use. Between 2.5 and 7.5 years of ART use was associated with a 5-7mg/dL increase in HDL-cholesterol (although this association was not significant for >10 years use). Both recruitment and time-updated PI use was significantly associated with all metabolic outcomes in univariable models: 12-24mg/dl increase with cholesterol outcomes (excluding HDL-cholesterol), and 49-52mg/dL with triglycerides. In contrast, both recruitment and time-updated PI-use were significantly associated with significant 5-7mg/dL decreases in HDL-cholesterol levels. NNRTI use at recruitment was associated ($p \leq 0.001$) with decreases in non-HDL cholesterol and fasting triglyceride, but increased HDL-cholesterol. Time-updated NNRTI was significantly associated with 11-22mg/dL increases in total, LDL, and non-HDL cholesterol: it was also associated with increased fasting triglyceride (30.8mg/dL, 95% CI: 9.8, 51.8). In comparison to PI-based HAART, NRTI mono-therapy or NNRTI-based HAART was significantly associated with 10-27mg/dL decrease in total, LDL, and non-HDL cholesterol: there was also a significant 49-52mg/dL increase with fasting triglyceride. Time updated NNRTI-based HAART was also significantly associated with an 8.1 mg/dL increase in HDL-cholesterol.

Multivariable multilevel modelling of total cholesterol

Recruitment LS was associated with an independent and significant increase in total cholesterol (21.2mg/dL, 95% CI: 11.0, 31.5) in the final model including recruitment factors (Table 7-8). Other variables that remained statistically significant, and that were associated with increased total cholesterol were maximum clinical condition B (12.0mg/dL, 95% CI: 1.2, 22.6), and recruitment PI (10.0mg/dL, 95% CI: 0.5, 19.5). However, recruitment age of 12-18 years, White ethnicity, and detectable viral load at recruitment, were all significantly associated with decreases in total cholesterol.

Table 7-8: Final multivariable multilevel model for total cholesterol: covariates at recruitment (*n* = 214)

		Estimate	95% confidence interval	*p - value*
Age*	**<12 years**	Comparison		
	12-18 years	-17.21	(-26.74, -7.68)	<0.001
Sex	**Male**	Comparison		
	Female	2.70	(-6.34, 11.75)	0.558
Ethnicity	**Black**	Comparison		
	White	-14.92	(-26.71, -3.13)	0.013
	Other	-19.02	(-42.24, 4.20)	0.108
Lipodystrophy syndrome*	**Absent**	Comparison		
	Present	21.23	(11.01, 31.45)	<0.001
Maximum clinical condition	**N/A**	Comparison		
	B	11.95	(1.31, 22.60)	0.028
	C	6.70	(-5.43, 18.82)	0.279
Detectable viral load*	**<50 copies/ml**	Comparison		
	≥50 copies/ml	-16.50	(-29.99, -3.01)	0.017
Protease inhibitors*	**No use**	Comparison		
	Use	10.01	(0.48, 19.54)	0.040
Duration of ART use*	**<2.5 years**	Comparison		
	2.5 - 4.9 years	-2.88	(-16.10, 10.33)	0.669
	5.0 – 7.4 years	-3.51	(-17.62, 10.60)	0.626
	7.5 – 9.9 years	-5.36	(-18.55, 7.84)	0.426
	≥10 years	-10.75	(-26.20, 4.71)	0.173
Intercept		186.32	(165.19, 207.44)	<0.001

***Variables at recruitment. Random effects parameters (standard deviation): clinical site (4.35, 95% CI: 0.93, 20.30), individual (28.84, 95% CI: 25.35, 32.58), with residual (23.3, 95% CI: 21.73, 24.98): 214 subjects clustered into 15 clinical sites with 607 observations.** See section "Investigating the random intercept of constructed models" on p283 for rationale on use of 3-level multi-level models

The increase in total cholesterol associated with time-updated LS (24.3mg/dL, 95% CI: 14.3, 34.3) was comparable to that seen with recruitment LS (Table 7-9). However, unlike the final recruitment model, time-updated PI was no longer statistically significant. Time-updated NNRTI was associated with an independent and significant increase in total cholesterol (19.5mg/dL, 95% CI: 5.4, 33.6). Both detectable viral load over follow-up, and recruitment age of 12-18 years were associated with decreases in total cholesterol.

Table 7-9: Final multivariable multilevel model for total cholesterol: time-updated covariates (*n* = 277)

		Estimate	95% confidence interval	*p - value*
Age at recruitment	**<12 years**	Comparison		
	12-18 years	-13.50	(-21.42, -5.58)	0.001
Sex	**Male**	Comparison		
	Female	1.68	(-5.79, 9.14)	0.660
Lipodystrophy syndrome*	**Absent**	Comparison		
	Present	24.30	(14.27, 34.33)	<0.001
Detectable viral load*	**<50 copies/ml**	Comparison		
	≥50 copies/ml	-18.27	(-29.42, -7.12)	0.012
Non-nucleoside reverse transcriptase*	**No use**	Comparison		
	Use	19.47	(5.35, 33.59)	0.007
Duration of ART use at recruitment	**<2.5 years**	Comparison		
	2.5 - 4.9 years	-5.00	(-16.14, 6.13)	0.379
	5.0 – 7.4 years	2.66	(-9.07, 14.40)	0.657
	7.5 – 9.9 years	-8.52	(-19.47, 2.44)	0.127
	≥10 years	-8.50	(-20.98, 3.97)	0.182
Intercept		167.57	(145.68, 189.45)	<0.001

***Time-updated variable. Random effects parameters (standard deviation): clinical site (0.00, 95% CI: 0.00, 0.00), individual (27.93, 95% CI: 25.11, 31.07), with residual (23.41, 95% CI: 22.03, 24.88): 277 subjects clustered into 15 clinical sites with 797 observations.** See section "Investigating the random intercept of constructed models" on p283 for rationale on use of 3-level multi-level models

Multivariable multilevel modelling of low density lipoprotein cholesterol

Older age and 7.5-9.9 years of ART use at recruitment were both associated with decreases in LDL-cholesterol (Table 7-10). In contrast recruitment LS was associated with an increase in LDL cholesterol (11.5mg/dL, 95% CI: 3.9, 19.1) in the final model. Other factors associated with an increase in LDL-cholesterol were recruitment PI use (8.3mg/dL, 95% CI: 1.1, 15.6) and a history of moderate clinical symptoms (9.5mg/dL, 95% CI: 1.2, 17.8).

Similar results were seen in the final model including time-updated covariates. The occurrence of LS over follow-up was associated with a significant increase in LDL cholesterol (14.5mg/dL, 95% CI: 5.9, 23.0) as illustrated in Table 7-11. Furthermore, time-updated PI use was also associated with increased concentrations (10.0mg/dL, 95% CI: 3.5, 16.6). Significant variables associated with a reduction in LDL-cholesterol included time-updated detectable viral load, recruitment age of 12-18, and recruitment duration of ART ≥10 years.

Table 7-10: Final multivariable multilevel model for low density lipoprotein (LDL) cholesterol at recruitment (*n* = 218)

		Estimate	95% confidence interval	*p - value*
Age*	**<12 years**	Comparison		
	12-18 years	-11.24	(-18.60, -3.87)	0.003
Sex	**Male**	Comparison		
	Female	3.84	(-3.17, 10.84)	0.283
Lipodystrophy syndrome*	**Absent**	Comparison		
	Present	11.49	(3.90, 19.09)	0.003
Maximum clinical condition	**N/A**	Comparison		
	B	9.51	(1.24, 17.78)	0.024
	C	7.24	(-2.08, 16.57)	0.128
Protease inhibitors *	**No use**	Comparison		
	Use	8.32	(1.09, 15.55)	0.024
Duration of ART use*	**<2.5 years**	Comparison		
	2.5 - 4.9 years	-0.76	(-10.88, 9.35)	0.882
	5.0 – 7.4 years	3.84	(-6.92, 14.61)	0.484
	7.5 – 9.9 years	-5.46	(-15.41, 4.50)	0.283
	≥10 years	-14.39	(-26.24, -2.54)	0.017
Intercept		89.64	(78.28, 100.99)	<0.001

***Variable at recruitment. Random effects parameters (standard deviation): clinical site (0, 95% CI: -), individual (22.6, 95% CI: 20.1, 25.5), with residual (18.3, 95% CI: 17.1, 19.7)): 218 subjects clustered into 15 clinical sites with 579 observations.** See section "Investigating the random intercept of constructed models" on p283 for rationale on use of 3-level multi-level models

Table 7-11: Final multivariable multilevel model for low density lipoprotein (LDL) cholesterol: time-updated covariates (*n* = 259)

		Estimate	95% confidence interval	*p - value*
Age*	**<12 years**	Comparison		
	12-18 years	-8.26	(-14.77, -1.74)	0.013
Sex	**Male**	Comparison		
	Female	2.93	(-3.34, 9.20)	0.360
Lipodystrophy syndrome*	**Absent**	Comparison		
	Present	14.49	(5.93, 23.04)	0.001
Detectable viral load*	**<50 copies/ml**	Comparison		
	≥50 copies/ml	-9.95	(-19.42, -0.47)	0.040
Protease inhibitors*	**No use**	Comparison		
	Use	10.03	(3.52, 16.55)	0.003
Duration of ART*	**<2.5 years**	Comparison		
	2.5 - 4.9 years	-3.23	(-12.35, 5.88)	0.487
	5.0 – 7.4 years	1.76	(-7.86, 11.38)	0.719
	7.5 – 9.9 years	-6.57	(-15.55, 2.41)	0.152
	≥10 years	-12.58	(-23.48, -1.68)	0.024
Intercept		97.36	(82.99, 111.74)	<0.001

***Time-updated variables. Random effects parameters (standard deviation): clinical site (1.89, 95% CI: 0.02, 194.74), individual (22.29, 95% CI: 19.90, 24.96), with residual (18.26, 95% CI: 17.09, 19.50): 259 subjects clustered into 15 clinical sites with 694 observations.** See section "Investigating the random intercept of constructed models" on p283 for rationale on use of 3-level multi-level models

Multivariable multilevel modelling of non-high density lipoprotein cholesterol

Recruitment LS was significantly associated with an independent increase in non-HDL cholesterol (20.9mg/dL, 95% CI: 12.0, 30.0) in the final model including recruitment covariates (Table 7-12). Other significant covariates included age at recruitment and ≥10 years of ART use at recruitment, both associated with a decrease in non-HDL cholesterol. Use of PI at recruitment was associated ($p < 0.01$) with an independent increase in non-HDL cholesterol of 17.7mg/dL (95% CI: 9.2, 26.2).

Variables included in the final model including time-updated variables differed from the model including recruitment covariates (Table 7-13). The increase in non-HDL cholesterol associated ($p < 0.001$) with LS occurring over follow-up was similar to that seen with recruitment LS being 21.7mg/dL (95% CI: 11.7, 31.6). Use of PI over the follow-up period was also associated with a significant and independent increase: 20.0mg/dL (95% CI: 12.4, 27.5). Time-updated variables associated with statistically significant reductions in non-HDL cholesterol included immunosuppression and detectable viral load (Table 7-13). Use of ART for ≥10 years at recruitment was also associated with a significant and independent decrease.

Table 7-12: Final multivariable multilevel model for non-high density lipoprotein (HDL) cholesterol at recruitment (*n* = 217)

		Estimate	95% confidence interval	*p - value*
Age *	**<12 years**	Comparison		
	12-18 years	-10.71	(-19.35, -2.08)	0.015
Sex	**Male**	Comparison		
	Female	-0.44	(-8.65, 7.78)	0.917
Lipodystrophy syndrome*	**Absent**	Comparison		
	Present	20.87	(11.95, 29.78)	<0.001
Maximum clinical condition	**N/A**	Comparison		
	B	9.71	(-0.01, 19.43)	0.050
	C	4.52	(-6.39, 15.42)	0.417
Protease inhibitors*	**No use**	Comparison		
	Use	17.67	(9.18, 26.16)	<0.001
Duration of ART *	**<2.5 years**	Comparison		
	2.5 - 4.9 years	-7.97	(-19.84, 3.91)	0.189
	5.0 – 7.4 years	-0.89	(-13.51, 11.73)	0.890
	7.5 – 9.9 years	-7.80	(-19.47, 3.86)	0.190
	≥10 years	-16.58	(-30.45, -2.72)	0.019
Intercept		105.96	(92.64, 119.27)	<0.001

***Variable at recruitment. Random effects parameters (standard deviation): clinical site (0.00, 95% CI: 0.00, 0.11), individual (27.0, 95% CI: 24.00, 30.44), with residual (19.81, 95% CI: 18.41, 21.33)): 217 subjects clustered into 15 clinical sites with 580 observations.** See section "Investigating the random intercept of constructed models" on p283 for rationale on use of 3-level multi-level models

Table 7-13: Final multivariable multilevel model for non-high density lipoprotein (HDL) cholesterol: time-updated covariates (*n* = 258)

		Estimate	95% confidence interval	*p - value*
Age at recruitment	**<12 years**	Comparison		
	12-18 years	-6.69	(-14.21, 0.84)	0.082
Sex	**Male**	Comparison		
	Female	-0.95	(-8.19, 6.29)	0.798
Lipodystrophy syndrome*	**Absent**	Comparison		
	Present	21.66	(11.74, 31.59)	<0.001
Any immuno-suppression*	**None**	Comparison		
	Any	-9.28	(-16.84, -1.72)	0.016
Detectable viral load*	**<50 copies/ml**	Comparison		
	≥50 copies/ml	-16.38	(-27.76, -5.00)	0.005
Protease inhibitors*	**No use**	Comparison		
	Use	19.96	(12.41, 27.50)	<0.001
Duration of ART use at recruitment	**<2.5 years**	Comparison		
	2.5 - 4.9 years	-10.58	(-21.15, 0.00)	0.050
	5.0 – 7.4 years	-3.43	(-14.54, 7.78)	0.545
	7.5 – 9.9 years	-9.81	(-20.22, 0.61)	0.065
	≥10 years	-12.84	(-25.47, -0.21)	0.045
Intercept		120.80	(103.39, 138.20)	<0.001

***Time-updated variable. Random effects parameters (standard deviation): clinical site (3.75, 95% CI: 0.82, 17.1), individual (25.90, 95% CI: 23.22, 28.89), with residual (19.90, 95% CI: 18.64, 21.25): 258 subjects clustered into 15 clinical sites with 695 observations.** See section "Investigating the random intercept of constructed models" on p283 for rationale on use of 3-level multi-level models

Multivariable multilevel modelling of high density lipoprotein (HDL) cholesterol

In contrast to previous models where LS at recruitment was significantly associated to total, LDL and non-HDL cholesterol, LS was non-significantly associated with increased HDL cholesterol (Table 7-14): 2.1mg/dL (95% CI: -2.0, 6.2, p = 0.320). Only two covariates were significantly associated with a decrease: -4.9mg/dL (95% CI: -8.7, -1.0) with age at recruitment of 12-18 years, and -8.7mg/dL (95% CI: -13.4, -4.0) with White ethnicity (compared to Black ethnicity). Female sex was associated with a significant increase in HDL-cholesterol of 8.0mg/dL (95% CI: 4.2, 11.9): this was the only outcome where female sex was associated with a significant and independent positive association. Other significant variables in the final model were associated with increases included NNRTI use, and 2.5-4.9 years duration of ART.

The occurrence of LS over follow-up was also non-significantly (p = 0.267) associated with an increase in HDL-cholesterol (2.7mg/dL, 95% CI: -2.0, 7.3) in the final model with time-updated covariates (Table 7-15). Female sex (4.9mg/dL, 95% CI: 1.6, 8.3), NNRTI-use over follow-up (7.7mg/dL, 95% CI: 1.3, 14.1), and increasing duration of ART use at recruitment (6.4mg/dL with ≥10 years, 95% CI: 0.5, 12.3), were all significantly associated with increased HDL-cholesterol. Time-updated variables associated ($p \leq 0.017$) with decreases in HDL-cholesterol included PI-use (-9.9mg/dL, 95% CI: -13.7, -6.1), and detectable viral load (-6.5mg/dL, 95% CI:

-11.8, -1.2). Other independent factors significantly associated with a decrease were age 12-18 years at recruitment, and White ethnicity.

Table 7-14: Final multivariable multilevel model for high density lipoprotein (HDL) cholesterol at recruitment (*n* =220)

		Estimate	95% confidence interval	*p - value*
Age*	**<12 years**	Comparison		
	12-18 years	-4.87	(-8.70, -1.04)	0.013
Sex	**Male**	Comparison		
	Female	4.76	(1.07, 8.46)	0.011
Ethnicity	**Black**	Comparison		
	White	-8.69	(-13.41, -3.97)	<0.001
	Other	-12.86	(-22.27, -3.44)	0.007
Lipodystrophy syndrome*	**Absent**	Comparison		
	Present	2.10	(-2.04, 6.24)	0.320
Non-nucleoside reverse transcriptase inhibitors*	**No use**	Comparison		
	Use	8.04	(4.18, 11.91)	<0.001
Duration of ART *	**<2.5 years**	Comparison		
	2.5 - 4.9 years	6.33	(0.92, 11.73)	0.022
	5.0 – 7.4 years	5.70	(-0.01, 11.41)	0.050
	7.5 – 9.9 years	1.95	(-3.43, 7.33)	0.478
	≥10 years	5.94	(-0.37, 12.15)	0.061
Intercept		51.82	(45.74, 57.90)	<0.001

***Variable at recruitment. Random effects parameters (standard deviation): clinical site (1.45, 95% CI: 0.08, 27.78), individual (12.27, 95% CI: 10.91, 13.80), with residual (8.59, 95% CI: 7.99, 9.23): 220 subjects clustered into 15 clinical sites with 590 observations.** See section "Investigating the random intercept of constructed models" on p283 for rationale on use of 3-level multi-level models

Table 7-15: Final multivariable multilevel model for high density lipoprotein (HDL) cholesterol: time-updated variables (*n* = 244)

		Estimate	95% confidence interval	*p - value*
Age at recruitment	**<12 years**	Comparison		
	12-18 years	-4.53	(-8.06, -1.00)	0.012
Sex	**Male**	Comparison		
	Female	4.94	(1.56, 8.32)	0.004
Ethnicity	**Black**	Comparison		
	White	-8.72	(-12.92, -4.52)	<0.001
	Other	-7.08	(-15.49, 1.33)	0.099
Lipodystrophy syndrome*	**Absent**	Comparison		
	Present	2.65	(-2.02, 7.32)	0.267
Detectable viral load*	**<50 copies/ml**	Comparison		
	≥50 copies/ml	-6.46	(-11.75, -1.17)	0.017
Protease inhibitors*	**No use**	Comparison		
	Use	-9.85	(-13.65, -6.06)	<0.001
Non-nucleoside reverse transcriptase*	**No use**	Comparison		
	Use	7.67	(1.26, 14.07)	0.019
Duration of ART use at recruitment	**<2.5 years**	Comparison		
	2.5 - 4.9 years	5.83	(0.88, 10.77)	0.021
	5.0 – 7.4 years	6.22	(0.92, 11.52)	0.021
	7.5 – 9.9 years	0.73	(-4.18, 5.64)	0.771
	≥10 years	6.41	(0.54, 12.29)	0.032
Intercept		58.92	(48.85, 68.99)	<0.001

***Time-updated variable. Random effects parameters (standard deviation): clinical site (1.22, 95% CI: 0.05, 30.49), individual (11.69, 95% CI: 10.41, 13.12), with residual (9.12, 95% CI: 8.52, 9.77): 244 subjects clustered into 15 clinical sites with 660 observations.** See section "Investigating the random intercept of constructed models" on p283 for rationale on use of 3-level multi-level models

Multivariable multilevel modelling of fasting triglyceride

Recruitment LS and PI use were both significantly associated with increased serum concentrations of fasting triglyceride in the final model including recruitment factors (Table 7 16). Significant and independent factors associated with a decrease in fasting triglyceride were female sex, and severely underweight BMI.

However, in the model with time-updated variables (Table 7-17), not only were the occurrence of LS and PI-use during follow-up significantly associated with increased fasting triglyceride, but White ethnicity was also associated (p = 0.001) with an increase (31.8mg/dL, 95% CI: 13.9, 49.8). Female sex was associated with an independent and significant decrease in fasting triglyceride.

Table 7-16: Final multivariable multilevel model for fasting triglyceride at recruitment (*n* = 219)

		Estimate	95% confidence interval	*p - value*
Age*	**<12 years**	Comparison		
	12-18 years	-0.27	(-18.13, 17.58)	0.976
Sex	**Male**	Comparison		
	Female	-20.77	(-36.78, -4.77)	0.011
Body mass index *	**Healthy weight**	Comparison		
	Severely underweight	-25.68	(-50.80, -0.55)	0.045
	Underweight	-1.44	(-20.57, 17.69)	0.883
	Overweight	-8.27	(-40.44, 23.90)	0.614
	Obese	-89.62	(-199.59, 20.34)	0.110
Lipodystrophy syndrome*	**Absent**	Comparison		
	Present	38.61	(20.72, 56.51)	<0.001
Protease inhibitors*	**No use**	Comparison		
	Use	43.64	(27.35, 59.93)	<0.001
Duration of ART*	**<2.5 years**	Comparison		
	2.5 - 4.9 years	-21.69	(-44.98, 1.62)	0.068
	5.0 – 7.4 years	-16.68	(-41.32, 7.95)	0.184
	7.5 – 9.9 years	-2.49	(-26.14, 21.16)	0.837
	≥10 years	-7.53	(-34.03, 18.97)	0.578
Intercept		70.24	(40.80, 99.69)	<0.001

***Recruitment variable. Random effects parameters (standard deviation): clinical site (0.00, 95% CI: 0.00, 0.17), individual (46.92, 95% CI: 40.35, 54.56), with residual (49.92, 95% CI: 46.18, 53.97): 219 subjects clustered into 15 clinical sites with 546 observations.** See section "Investigating the random intercept of constructed models" on p283 for rationale on use of 3-level multi-level models

Table 7-17: Final multivariable multilevel model for fasting triglyceride: time-updated variables (*n* = 245)

		Estimate	95% confidence interval	*p - value*
Age at recruitment	**<12 years**	Comparison		
	12-18 years	2.16	(-12.56, 17.89)	0.787
Sex	**Male**	Comparison		
	Female	-18.24	(-33.49, -2.99)	0.019
Ethnicity	**Black**	Comparison		
	White	31.84	(13.88, 49.79)	0.001
	Other	13.49	(-24.05, 51.03)	0.481
Lipodystrophy syndrome*	**Absent**	Comparison		
	Present	38.27	(17.60, 58.95)	<0.001
Protease inhibitors*	**No use**	Recruitment		
	Use	45.74	(29.82, 61.67)	<0.001
Duration of ART use at recruitment	**<2.5 years**	Comparison		
	2.5 - 4.9 years	-18.85	(-41.24, 3.54)	0.099
	5.0 – 7.4 years	-13.57	(-37.34, 10.21)	0.263
	7.5 – 9.9 years	3.75	(-18.43, 25.92)	0.741
	≥10 years	-2.00	(-28.09, 24.09)	0.881
Intercept		45.28	(17.12, 73.44)	0.002

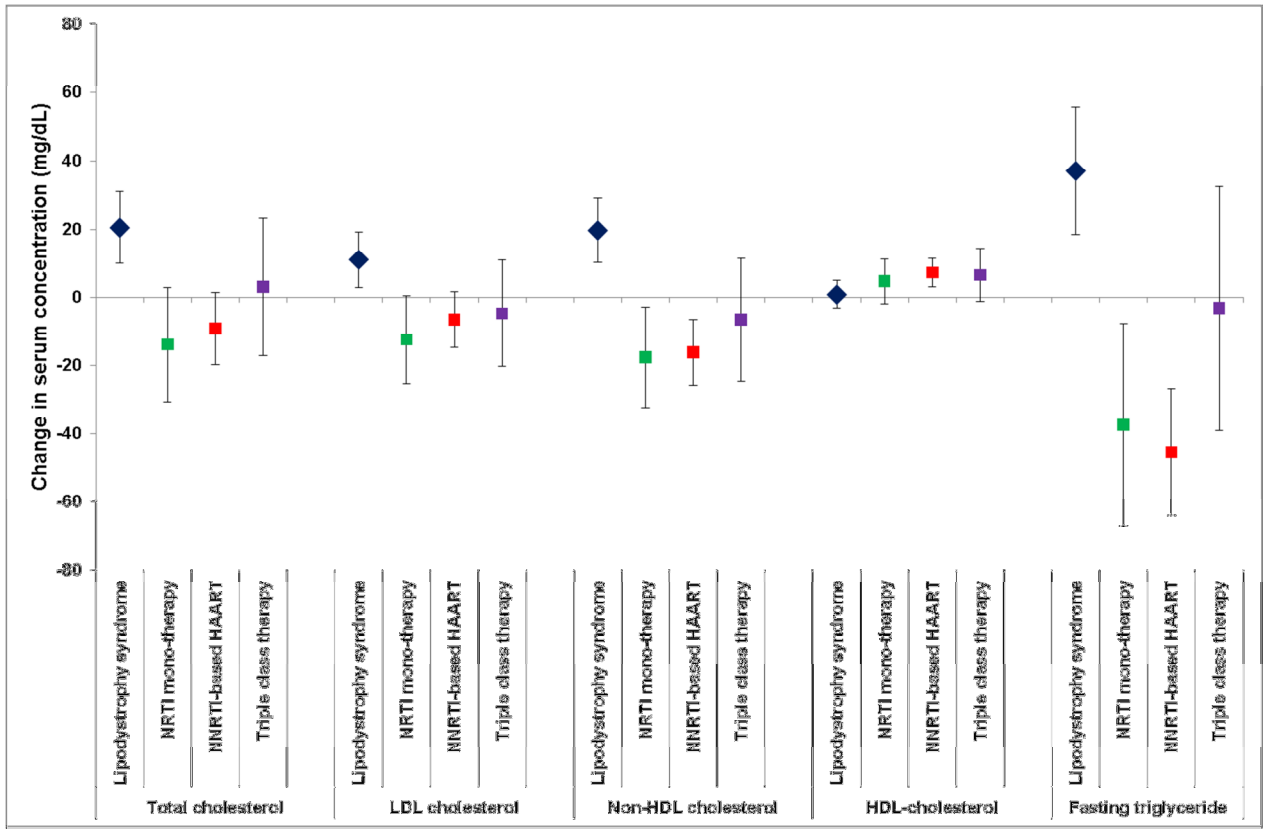
***Time-updated variables. Random effects parameters (standard deviation): clinical site (0.00, 95% CI: 0.00, 0.00), individual (49.28, 95% CI: 42.91, 56.60), with residual (49.81, 95% CI: 46.23, 53.65): 245 subjects clustered into 15 clinical sites with 608 observations.** See section "Investigating the random intercept of constructed models" on p283 for rationale on use of 3-level multi-level models

Examination of the effect of lipodystrophy syndrome and antiretroviral regimen

Further models were constructed to explore the independent effects of LS and specific ART regimens on changes in cholesterol and fasting triglyceride levels over follow-up. In intuitively adjusted models including recruitment explanatory variables, LS was significantly associated ($p \leq 0.008$) with increases in total cholesterol, LDL-cholesterol, non-HDL cholesterol and fasting hypertriglyceridemia (Figure 7-12). The increase seen with total cholesterol was 20.5mg/dL (95% CI: 10.0, 31.0), with LDL-cholesterol was 11.1mg/dL (95% CI: 2.9, 19.2), with non-HDL cholesterol was 19.7mg/dL (95% CI: 10.3, 29.1), and with fasting triglyceride was 37.0mg/dL (95% CI: 18.5, 55.5). In comparison to PI-based HAART, NRTI mono-therapy (-17.6mg/dL, 95% CI: -32.4, -2.9), and NNRTI-based HAART (-16.2mg/dL, 95% CI: -25.7, -6.6) were associated with decreases in non-HDL cholesterol. Similarly, both NRTI mono-therapy (-37.4mg/dL, 95% CI: -67.1, -7.8) and NNRTI-based HAART (-45.4mg/dL, 95% CI: -64.0, -26.8) were significantly associated with reduced fasting triglyceride. Of note only 29 subjects (8% of the cohort) were being treated with NRTI non-therapy, reflecting that this is not a standard treatment strategy. In contrast, a 7.3mg/dL (95% CI: 3.1, 11.4) increase in HDL-cholesterol was seen with NNRTI-based HAART ($p = 0.001$). Full models are presented in Table G-3 in Appendix G

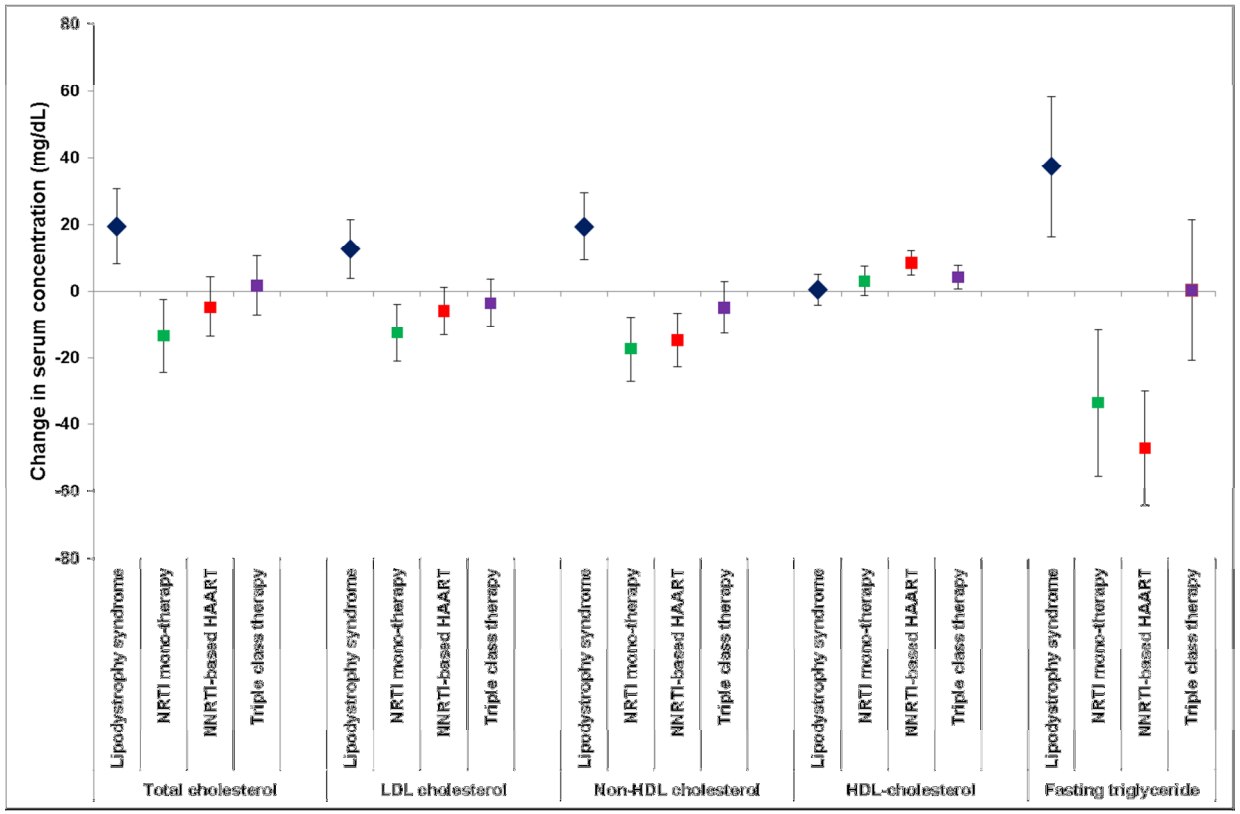
Similar results were seen in the model including time-updated variables (Figure 7-13). Time-updated LS was associated ($p \leq 0.005$) with increases in total cholesterol (19.4mg/dL, 95% CI: 8.2, 30.6), LDL-cholesterol (12.6, 95% CI: 3.8, 21.3), non-HDL cholesterol (19.3mg/dL, 95% CI: 9.3, 29.4), and fasting triglyceride (37.3mg/dL, 95% CI: 16.3, 58.2). A non-significant ($p = 0.824$) increase in HDL-cholesterol was also seen (0.5mg/dL, 95% CI: -4.1, 5.1). Specific time-updated ART regimens remained significant ($p \leq 0.023$) for all metabolic outcomes. Compared to PI-based HAART, NRTI mono-therapy was associated with decreases in total cholesterol (-13.4mg/dL, 95% CI: -24.4, -2.4), LDL-cholesterol (-12.5mg/dL, 95% CI: -21.0, -3.9), non-HDL cholesterol (-17.3mg/dL, 95% CI: -27.0, -7.7), and fasting triglyceride (-33.5mg/dL, 95% CI: -55.5, -11.6). In addition, NNRTI-based HAART was associated ($p < 0.001$) with decreases in non-HDL cholesterol (-14.7mg/dL, 95% CI: -22.7, -6.6) and fasting triglyceride (-47.1mg/dL, 95% CI: -64.4, -29.8). In contrast, significant increases in HDL cholesterol were seen with time-updated NNRTI-based HAART (8.4mg/dL, 95% CI: 4.7, 12.1) and triple-class therapy (4.2mg/dL, 95% CI: 0.7, 7.7), in comparison to time-updated PI-based HAART. Full details of each model are presented in Table G-5 of Appendix G.

Figure 7-12: Estimated change in serum concentrations during follow-up by adjusted multivariate multilevel models including recruitment covariates



Comparison group for ART regimen is PI-based HAART. Models are 3-level containing random intercept with subjects clustered within clinical site. Fixed part of models adjusted for sex, ethnicity, maximum clinical status, and variables at recruitment: age, BMI, immunosuppression, detectable viral load, and duration of ART use. See Table G-3 and Table G-4 in Appendix G for full models. See section "Investigating the random intercept of constructed models" on p283 for rationale on use of 3-level multi-level models

Figure 7-13: Estimated change in serum concentrations during follow-up by adjusted multivariable multilevel models including time-updated variables



Comparison group for ART regimen is PI-based HAART. Models are 3-level containing random intercept with subjects clustered within clinical site. Fixed part of models adjusted for sex, ethnicity, maximum clinical status, and time-updated variables: age, BMI, immunosuppression, detectable viral load, and duration of ART use. See Table G-5 and Table G-6 in Appendix G for full models. See section "Investigating the random intercept of constructed models" on p283 for rationale on use of 3-level multi-level models.

Investigating the random intercept of constructed models

In order to validate the use of a 3-level multivariable model (repeated measures in individuals clustered in clinical sites) over a 2-level (repeated measures in individuals) or null (no random intercept, i.e. linear regression model), log likelihood tests were conducted.

Tests were conducted to compare the 3-level models with 2-level models: all stepwise-devised models including recruitment covariates showed no significant differences between the 2 and 3-level models ($p \geq 0.493$), indicating that the addition of the extra parameter in the random intercept did not improve the model (Appendix G: Table G-7). This was also true of the intuitively adjusted multivariable model for non-HDL cholesterol including ART regimen at recruitment ($p > 0.999$). All other models including recruitment explanatory variables showed a significant ($p < 0.001$) difference between 2-level and 3-level models, indicating inclusion of individuals clustered in the clinical site in the random intercept improved the fit of the model.

In contrast, log ratio tests comparing 3-level models and null models (no random intercept) showed significant differences ($p < 0.001$) for all previously derived recruitment and time-updated models (Appendix G: Table G-8): thus inclusion of the random intercept has improved all models.

All multilevel models presented in this chapter are 3-level in order to show consistent and comparable models.

QQ plots were constructed to examine the normal assumption in level-2 and level-3 residuals. All plots demonstrated that the normal assumption had not been violated.

7.3.6 Management of lipodystrophy syndrome

Data on management of LS was collected for 207 subjects in total, of whom 59% ($n = 123$) were receiving an intervention: 10.4% ($n/N = 18/155$) had pharmacological intervention (including use of omega 3 fatty acid, testosterone, other steroids, statins and growth hormones), 33.5% ($n/N = 91/181$) had dietary intervention, and 26.5% ($n/N = 72/200$) had physical activity intervention. Table 7-18 summarizes the distribution of management interventions in relation to LS symptoms at the beginning and at the end of follow-up. There was no significant difference in the proportion of subjects managed by each management strategy (for LS, any body fat alterations or any metabolic abnormality) by country ($p \geq 0.136$)

The most common management approach to LS at recruitment was dietary and physical activity, with one quarter ($n = 41$) of children being managed in this way. The second most common approach was dietary intervention only (19%, $n = 30$), followed by physical activity alone (8.9%, $n = 14$). This pattern was seen with both recruitment body fat alterations and

recruitment metabolic abnormality at recruitment (and also with all outcomes at the end of follow-up). The proportion of subjects who had body fat alterations and were undergoing physical activity intervention alone increased (p = 0.009) between recruitment (9%, n = 11) and the end of follow-up (17%, n =19). Although there were significant differences in the proportion of subjects with specific LS symptoms and undergoing particular interventions between recruitment and the end of follow-up (Table 7-18), numbers were small.

Management strategy by lipodystrophy symptoms

Among the 12 subjects with body fat alterations at recruitment and who had their LS managed by pharmacological intervention (Figure 7-14), treatment was most common in subjects with lipoatrophy alone or together with lipohypertrophy (n = 9). Of the 47 children and adolescents with fat alterations at recruitment who were managed with physical activity, most had both lipoatrophy and lipohypertrophy (38.3%, n =1 8) or lipohypertrophy alone (36.2%, n = 17). Of 63 children managed with dietary intervention for initial body fat alterations, 42.9% (n =2 7) had both lipoatrophy and lipohypertrophy, while 36.5% (n = 23) had lipohypertrophy (p = 0.020). No statistical association ($p \geq 0.05$) was seen between severity of lipoatrophy or lipohypertrophy and management by diet, nor between severity of lipohypertrophy and management by physical activity. However, increasing severity of lipoatrophy was associated with use of physical activity as an intervention with such management in 19% (n/N = 9/47) of subjects with mild, 34% (n/N = 10/29) with moderate, and 59% (n/N = 10/17) with severe symptoms (p = 0.009),

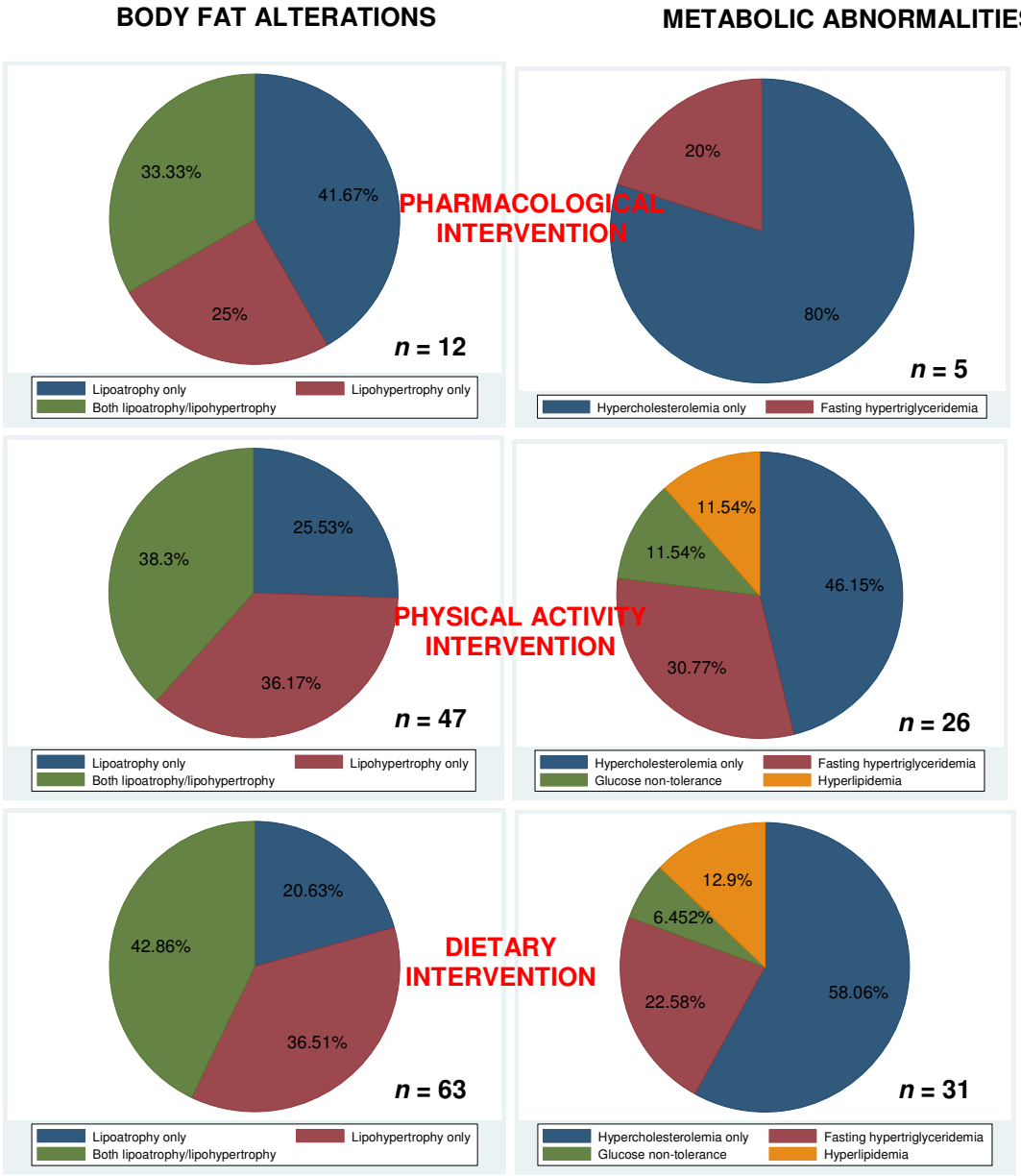
Management with pharmacological, physical activity or dietary intervention (Figure 7-14) was more common among subjects with hypercholesterolemia as their only manifestation of dyslipidemia, at 80.0% (n=4), 46.2% (n=12), and 58.1% (n=18) respectively. The second most common manifestation was fasting hypertriglyceridemia alone, treated with pharmacology (20%, n=1), physical activity (30.8%, n=8), and dietary intervention (22.6%, n=7).

Table 7-18: **Distribution of subjects with specific lipodystrophy symptoms being managed with particular interventions at recruitment and end of follow-up symptoms**

	Body fat alterations		Metabolic abnormality		Lipodystrophy syndrome	
	Recruitment	End of follow-up	Recruitment	End of follow-up	Recruitment	End of follow-up
No intervention	40 (*32.8*)	29 (*25.7*)	25 (*41.7*)	35 (*43.8*)	58 (*37.1*)	56 (*35.0*)
Pharmacological intervention only	6 (*4.9*)	5 (*4.4*)	2 (*3.3*)**	0**	6 (*3.8*)	5 (*3.1*)
Dietary intervention only	25 (*20.5*)	24 (*21.2*)	10 (*16.7*)	13 (*16.3*)	30 (*19.0*)	33 (*20.6*)
Physical activity only	11 (*9.0*)**	19 (*16.8*)**	3 (*5.0*)	10 (*12.5*)	14 (*8.9*)	20 (*12.5*)
Pharmacological and dietary intervention	3 (*2.5*)	3 (*2.7*)	0	0	3 (*1.9*)*	4 (*2.5*)
Pharmacological and physical activity intervention	1 (*0.8*)	1 (*0.9*)	1 (*1.7*)*	0*	2 (*1.3*)	1 (*0.6*)
Dietary and physical activity intervention	33 (*27.1*)	29 (*25.7*)	17 (*28.3*)	18 (*22.5*)	41 (*26.0*)	37 (*23.1*)
Pharmacological, dietary and physical activity intervention	2 (*1.6*)	3 (*2.7*)	2 (*3.3*)	4 (*5.0*)	3 (*1.9*)	4 (*2.5*)

Percentages are given in brackets χ^2 tests used to compare proportions of subjects with specific lipodystrophy syndromes and particular managements strategies at recruitment and the end of follow-up: * $p < 0.05$, $p < 0.01$, *** $p < 0.001$**

Figure 7-14: Initial symptoms of lipodystrophy syndrome by management intervention



Specific management by pharmaceutical strategies

Specific drug interventions were known for 14 subjects (Table 7-19): 2 were treated with omega 3 fatty acids, 2 with testosterone, 4 with steroids, 1 with statin, and 5 with growth hormone. Details on duration of treatment by LS management interventions were not collected.

Table 7-19: Subjects with details on specific pharmaceutical management of lipodystrophy syndrome (*n* = 14)

	Age * (years)	Drug	Lipodystrophy status	
			Initial	End of follow-up
Patient 1	9.9	Omega 3 fatty acid	Metabolic abnormality alone	No symptoms
Patient 2	11.3		Both**	Both
Patient 3	16.6	Testosterone	Body fat alterations only	Body fat alterations only
Patient 4	15.6		Both	Body fat alterations only
Patient 5	15.0	Steroid	Body fat alterations only	No symptoms
Patient 6	15.9		Body fat alterations only	Body fat alterations only
Patient 7	16.1		Body fat alterations only	Body fat alterations only
Patient 8	4.9		Body fat alterations only**	Body fat alterations only
Patient 9	9.6	Statin	Both	Both
Patient 10	14.4	Growth hormone	Body fat alterations only	Body fat alterations only
Patient 11	13.3		Body fat alterations only	Body fat alterations only
Patient 12	12.1		Body fat alterations only	Metabolic abnormality only
Patient 13	9.8		Both	No symptoms
Patient 14	3.2		Body fat alterations only**	Body fat alterations only

Initial lipodystrophy status is status at recruitment unless otherwise stated. Both refers to symptoms of body fat alterations and metabolic abnormality occurring concurrently. *Age at recruitment. **Incident case at second study visit

Among these 14 subjects, 5 experienced changes in LS symptoms over the follow-up period. Patient 1 no longer had any metabolic abnormality at the end of recruitment, and remained free of any body fat abnormalities. Patient 4, treated with testosterone, had both body fat alterations and metabolic abnormality at recruitment but only had body fat alterations at the end of follow-up. Patient 5, treated with (unspecified) steroids, had body fat alterations at recruitment, but had no LS symptoms at the end of follow-up. Of the 5 subjects treated with growth hormone, 2 experienced changes in LS symptoms over the follow-up period: Patient 13 had complete regression of LS, with neither body fat alterations nor metabolic abnormality at the end of follow-up, while Patient 12 had body fat alterations at recruitment, but had developed metabolic abnormality (without body fat alterations) by the end of the study period.

7.4 Key points

- Subjects with incident body fat alterations had non-significant increases in levels of fasting triglyceride, total cholesterol, LDL-cholesterol and non-HDL cholesterol, with non-significant decreases in HDL-cholesterol over the follow-up period.

- Significant increases in the proportion of subjects with body fat alterations over follow-up were seen in the children and adolescents who developed incident metabolic abnormality

- Metabolic abnormality at recruitment was an independent risk factor for incident body fat alterations in Cox proportional hazards models, associated with a significant increased risk. No statistically significant association was seen between (either recruitment or time-updated) body fat alterations and incident metabolic abnormality.

- Both recruitment and time-updated LS was significantly associated with increased total cholesterol, LDL-cholesterol, non-HDL cholesterol and fasting triglyceride levels in prospective multivariable multilevel analyses No significant association was found with HDL-cholesterol.

- Both recruitment and time-updated PI use was independently associated with an increase in LDL-cholesterol, non-HDL cholesterol and fasting triglyceride ($p < 0.05$), after adjusting for LS. Furthermore, in comparison to PI-based HAART use at recruitment, both NRTI mono-therapy and NNRTI-based HAART were associated with independent and significant decreases in total cholesterol, LDL-cholesterol, non-HDL cholesterol, and fasting triglyceride: similar results were seen for non-HDL cholesterol and fasting triglyceride in analyses investigating time-updated ART regimens.

- NNRTIs were significantly associated with increased HDL-cholesterol, following adjustment for LS: this was true of both NNRTI use at recruitment or at any time during follow-up. Moreover, significant increased HDL-cholesterol was associated with (recruitment and time-updated) NNRTI-based HAART (in comparison to PI-based HAART) in prospective multivariable modelling. An independent and significant increase in HDL-cholesterol was also seen with triple-class therapy use during follow-up.

- Older age was significantly associated with decreases in all cholesterol outcomes in prospective multivariable multilevel models, and maximum clinical status was associated with decreases in total, LDL and non-HDL cholesterol.

- Statistically significant factors associated with HDL-cholesterol over follow-up were White compared to Black ethnicity (negative association) and female sex compared to male sex (positive association).

- Almost 60% of the 207 subjects who had LS-management data available were being treated with a specific intervention. The most common strategy employed to manage symptoms of LS were lifestyle modification by physical activity or nutritional interventions.

- Only 10% of subjects with LS (and who had management data available) were treated with pharmaceutical intervention: the most common approach was growth hormone use, followed by steroid use. Indicators for treatment by a specific strategy were unclear.

8. Discussion

8.1 Introduction

Cross sectional estimates of LS prevalence in HIV-infected adults as high as 83% have been estimated [226], with the association of LS with ART being well established in many adult studies[199,215,235,393-398]. Fewer studies have investigated the syndrome in children: generally these have been smaller and limited to a cross-sectional design. Nevertheless, study of paediatric LS is of increasing relevance as greater numbers of HIV-infected children gain access to ART[60], and accumulate longer durations of exposure to these drugs.

This is the largest, multicentre, prospective study designed specifically to investigate both body fat alterations and metabolic abnormality in children and adolescents. This thesis has explored the phenotype of LS in children, estimated both prevalence and incidence of multiple manifestations of LS, and used both cross sectional and prospective analyses to identify associated risk factors in subjects aged <18 years. Statistical models have been fitted to investigate how LS affects serum concentrations of cholesterol and triglyceride. Finally it describes strategies used by clinicians to manage LS symptoms in patients.

The pathogenesis of LS and biological plausibility of HIV and ART as causal agents are discussed in Chapter 1: additional proposed biological mechanisms are introduced in this chapter. The effects of these causal agents on LS outcomes were investigated in cross-sectional analyses in Chapters 4 and 5, and in longitudinal analyses in Chapters 6 and 7. The results and consequent inferences from this thesis are reviewed in the context of the published literature in Section 8.2, establishing consistency with other studies. Furthermore, this chapter discusses novel findings from this study in the context of the published literature. Strength of the association between risk factors and outcomes are discussed in Section 8.3 of this chapter. Possible confounding factors are discussed in Section 8.2 and the measures used to address them are explained in Section 8.3. The possible role of chance in the analyses is discussed in Section 8.3. The strength of the evidence of the research presented in this thesis is judged within this framework.

8.2 Main findings

This is a prospective observational study that followed-up 426 HIV-infected children aged 2-18 years for a median of 4.2 years. The prevalence of LS at recruitment was 56.5%, which increased to 61.6% at the end of follow-up. Incidence was 17.8 per 100 person-years. Significant risk factors for LS included White ethnicity, increasing BMI, PI-use and NNRTI-use (and specifically efavirenz), and stavudine-use (all associated with increased risk). Indeed, while LS, PI-use, maximum clinical status was significantly associated with increases in serum concentrations of >1 of LDL-cholesterol/non-HDL-cholesterol/LDL-cholesterol,/fasting triglyceride, older age was significantly associated with decreases. NNRTI-use and female sex was significantly associated with increased HDL-cholesterol concentrations; Black ethnicity was significantly associated with a decrease. The most frequent approach to managing symptoms of LS in this cohort was lifestyle modification by nutritional modification or recommendation of physical activity.

Comparison of results with other LS-studies is problematic due to the diversity in study design and population, including differences in definitions and assessments of LS. Table H-1 in Appendix H summarizes the major adult studies cited in this thesis (excluding clinical trials). Notwithstanding overlapping study populations between investigations, the vast majority of previous adult studies have been conducted in the developed world, with only 3 conducted in Africa (2 in Rwanda[193,399] and 1 in Benin[400]), and 2 in Asia (1 in Singapore[401] and 1 in India[227]). Several studies were of prospective design ranging from 3-months[402] to 101 weeks[403], with one study utilizing 5 years of data in a historical cohort[398]: only 1 prospective study was conducted in the developing world[400]. However, the greatest heterogeneity between studies was seen in definitions of LS. While the largest studies ($n \geq 925$)[215,394,404,405] have relied on clinician assessed lipoatrophy and lipohypertrophy, a 1-year prospective study of 745 Canadian resident patients relied on patient-assessed fat alterations[406], and a cross-sectional US study of 416 patients calculated indices of alterations from circumference (mid-arm, mid-thigh, and mid-waist), with skinfold (subscapular, mid-thigh, triceps and abdomen skinfold) measures[236]. Similarly, thresholds in the definition of hyperlipidemia have varied between studies: hypercholesterolemia was defined as total cholesterol levels ≥193mg/dL[225] to ≥250mg/dL[407], while hypertriglyceridemia was defined as triglycerides ≥150mg/dL[227,408] to ≥531mg/dL[409]. However, other studies used definition reliant on HDL-cholesterol, LDL-cholesterol and fasting triglyceride thresholds[193,216,225,227,394,402,406,408,410-412], Similar heterogeneity is seen in investigations of LS in HIV-infected children: (Table 8-1 and Table 8-2). Within this study, body fat alterations were clearly defined by severity at specific locations, and metabolic abnormality according to age and sex-defined thresholds of cholesterol and fasting triglyceride. The approaches taken by other individual paediatric studies will be discussed in the context of their results.

8.2.1 Prevalence and incidence

The estimates of prevalence of body fat alterations, both at recruitment and at the end of follow-up, were substantially higher than the 18-33% reported by previous studies (Table 8-1). Of note, all but one study had less than 60 participants. Crucially, samples in previous paediatric studies were younger compared to this cohort (median age: 12.2 years). It has been argued that the development of LS is a graded and progressive process, while the natural development of adipose tissue in children may have a protective effect which wanes during and after puberty[194]. Moreover, given ART is life-long, older children are more likely to have longer durations of exposure to drugs, and furthermore, exposure to older toxic drugs commonly used before the introduction of newer, more benign formulations. Thus, the comparatively older ages of subjects in this cohort may help to explain the greater prevalence observed.

While adult studies have not found the pattern in proportions of type of body fat alterations seen here (i.e. similar proportions of subjects with lipohypertrophy, lipoatrophy, and the combined phenotype), they have reported higher prevalences[401,413]. Similar to baseline prevalences seen in this cohort, prevalence of lipoatrophy-only was 15%, of lipohypertrophy-only was 12%, and of mixed lipoatrophy and lipohypertrophy was 10% in 581 HIV-infected adults enrolled in the multi-centre French Aquitaine Cohort, with significant differences seen in the prevalence of lipoatrophy-only between the genders[216]. Indeed, in the current study, significant differences were seen in the prevalence of lipohypertrophy in the trunk and the neck between sexes with higher proportions seen in males compared to females. However, analysis of 1348 adults attending 15 clinical centres in Australia resulted in estimated lipoatrophy-only prevalence of 20%, lipohypertrophy-only of 6%, and combined lipoatrophy and lipohypertrophy of 27%[405]. Similarly, among 194 subjects with body fat alterations in a Rwanda/South Africa based study the proportion of subjects with concurrent lipohypertrophy and lipoatrophy was highest (three-quarters), followed by lipohypertrophy alone (one in twenty), and then lipoatrophy alone (one in ten)[193]. Thus, while this study and previous paediatric investigations suggest the prevalence of body fat alterations does increase from adolescence into adulthood, there is conflicting evidence as to whether the manifestation of the concurrent lipoatrophy and lipohypertrophy phenotype also occurs more frequently.

Among subjects with body fat alterations in this cohort, the most common location was the trunk, followed by the face, legs, arms and buttocks, with the least common locations being the neck and breast. This pattern is similar to that seen in studies investigating adult populations[216,226], and identical to that seen in 124 children with body fat alterations in a previous European Cohort[209].

Table 8-1: Estimated prevalence of body fat alterations in published paediatric studies

Study	Study type	Location	Number of subjects	Age	Assessment of body fat alterations	Definition of body fat alteration	Estimated prevalence
Amaya *et al*, (2002)[195]	Cross-sectional	USA	40	9.1†	Investigator	Fat wasting in extremities, buttocks and face. Fat accumulation in the abdomen and dorsocervical spine	18
Arpadi *et al*, (2001)[200]	Cross-sectional	USA	28	4.3 – 12.2†††	DEXA scan	Both decrease in arm and leg fat and an increase in trunk fat, by repeated scanning	29
Bockhorst *et al*, (2003)[202]	Prospective 6-month follow-up	USA	26		Not disclosed	Fat wasting in the face, arms or legs and/or fat accumulation (central obesity or breast hypertrophy)	23.1
European Paediatric Lipodystrophy Cohort (2004)[209]	Cross-sectional	Multi-country Europe	477	9.78††	Clinician	Lipoatrophy: sunken cheeks/sunken eyes/ prominent zygomatic arch on face. Skinny/prominent veins/prominent muscularity or bones in limbs. Buttocks have loose skin folds, prominent muscles/loss of contouring/hallowing. Lipohypertrophy: increased abdominal girth/ buffalo hump/enlarged breast	26.0 (22.1, 30.2)**
Jacquet *et al.*(2000)[194]	Cross-Sectional	France	39	9.1†	Not disclosed	Peripheral fat wasting: facial and/or buttock and/or limp atrophy with arm skinfold thickness <3rd percentile of the reference values for sex and age.* Truncal adiposity: breast enlargement and/or buffalo neck and/or relative abdominal obesity with trunk:arm skinfold ratio >2 standard deviations	33.3
Sanchez-Torres *et al*. (2005)[207]	Cross-sectional	Spain	57	9.5†	Clinician	Lipoatrophy: sunken cheeks and thin extremities with prominent veins with/without buttock atrophy Lipohypertrophy: increased abdominal girth and breast enlargement with/without buffalo hump and/or limpomas	25.0 (14.8, 34.6)**

In subgroup analyses of children and adolescents with body fat alterations in this study, subjects with concurrent lipoatrophy and lipodystrophy had higher median fasting triglyceride than either those subjects with lipoatrophy-alone or with lipohypertrophy-alone. It has been suggested that the occurrence of concurrent lipoatrophy and lipohypertrophy occurs as the endpoint of systematic pathogenesis involving progressive metabolic changes and insulin resistance[209,414]. This theory is supported by significant trends in metabolic outcomes being seen with increasing severity of fat disturbance[405], and both normal and pathological insulin sensitivity observed in HIV-infected patients with impaired glucose tolerance[397]. Results from this thesis endorse this hypothesis as subjects with both types of body fat alterations demonstrated metabolic changes characteristic of insulin resistance.

Female subjects with body fat alterations had significantly increased standardized median total cholesterol, and standardized median (pro-atherosclerotic) non-HDL cholesterol, compared to female subjects without alterations. Male subjects without body fat alterations had significantly higher levels of (anti-atherosclerotic) HDL-cholesterol compared to males with body fat alterations. Several pro-atherosclerotic pathways stem from alterations in the ratio of HDL-cholesterol to LDL-cholesterol[415], which have implications in established inflammatory cascades implicated in CVD pathogenesis[416]. Thus children who were symptomatic with body fat alterations had also developed an unhealthy metabolic profile, regardless of whether they had manifest hyperlipidemia.

Table 8-2 illustrates the prevalence of hypercholesterolemia, hypertriglyceridemia, and hyperlipidemia in previously published paediatric studies. Only two previous studies employed age and gender thresholds in their definitions of hyperlipidemia[196,208], while the remainder use thresholds which were consistent between sexes and age groups. For example, prospective data from 2122 children and adolescents enrolled in the PACTG 219C reported a prevalence of hypercholesterolemia of 26% using a threshold of 220mg/dL[197], while a much higher prevalence of 53.4% was reported in a USA-based retrospective cohort of 178 children using the lower threshold of 200mg/dL[359]. Nevertheless, the estimated prevalence of hypercholesterolemia at the end of follow-up in the current study (13.3%) is similar to the (age and gender defined) prevalence of hypercholesterolemia (13.0%) reported in an earlier analysis from the PACTG 219C study[196]. Moreover, the finding that hypertriglyceridemia occurs more frequently than hypercholesterolemia here is consistent with previous studies[201,203,206,208], although the converse has also been reported[195].

Table 8-2: Estimated prevalence of metabolic abnormality in published paediatric studies

Study	Study type	Location	Number of subjects	Age (years)	Definition of metabolic abnormality	Estimated prevalence
Amaya *et al*, (2002)[195]	Cross -sectional	USA	40	9.1†	HC: cholesterol > 170mg/dL HT: triglyceride >135mg/dL	HC: 68 HT: 28
Aurpibul *et al* , (2007)[201]	Prospective 144 weeks follow-up	Thailand	90	7.6† (at enrolment)	HC: total cholesterol >200mg/dL and LDL cholesterol ≥130mg/dL HT: triglyceride >200mg/dL	HC: 11 Fasting HT: 12
Carter *et al*, (2006)[203]	Prospective <7 years follow-up	USA (multicentre)	178	6.3† (at enrolment)	HC: cholesterol ≥200mg/dL HT: triglyceride ≥ 150mg/dL	HC: 47 HT: 67
Desai *et al* (2008)[204]	Prospective 18 month follow-up	USA	48	6-15†††	HL: cholesterol ≥ 200mg/dL and/or triglycerides ≥ 150mg/dL	HL: 58
European Paediatric Lipodystrophy Cohort (2004)[209]	Cross-sectional	Multi-country European	477	9.78††	HC: cholesterol ≥ 200mg/dL HT: triglyceride ≥ 150mg/dL	HC: 21 HT: 27
Farley *et al* (2005)[196]	Prospective follow-up	USA	1812	4-19†††	HC: cholesterol >95th percentile from NHANES III standards (by age, race and gender)	HC: 13.0
Jaquet *et al*, (2000)[194]	Cross-sectional	France	29	9.1†	HC: cholesterol > 95th percentile	HC: 17.9
Kim *et al*, (2009)[359]	Retrospective cohort 10 year follow-up	USA	178	2.74† (at presentation)	HC: cholesterol > 200mg/dL	HC: 53.4
Parakh *et al*, (2009)[206]	Cross-sectional	India	52	6.0††	HC: cholesterol ≥ 200mg/dL HT: triglycerides ≥ 150mg/dL	HC: 7.7 HT:19.2
Dos Reis *et a*l (2011)[358]	Cross-sectional	Brazil	119	11.9†	HC: cholesterol ≥ 150mg/dL HT: triglycerides ≥ 100mg/dL	HC: 33.9 HT: 35.6
Solórzano Santos *et al*, (2006)[208]	Prospective 27 month follow-up	Mexico	24	<17 years†††	HC: cholesterol > 200mg/dL HT: gender and age defined thresholds	HC: 62.5 HT: 79.2
Tassiopoulos *et a*l, (2008)[197]	Prospective follow-up	USA	2122	9.21†	HC: cholesterol ≥ 220mg/dL	HC: 26

Several studies in adults have reported higher prevalences of hyperlipidemia with prevalences of hypercholesterolemia of ≤57% and of (fasting and non-fasting) hypertriglyceridemia ≤52% in both developed and less-developed countries[225,411 394]. Thus it is likely that pathogenesis of metabolic abnormality may be on-going as duration of ART use, or HIV-infection, increases, concurrent with normal increases in plasma lipids that occur from childhood into adolescence and, from a post-pubertal trough into early adulthood (cholesterol), and from childhood to early adulthood (triglyceride)[381,417-419].

The incidence of body fat alterations was 8.01 per 100 person-years here, where follow-up averaged 4 years, which was similar to that reported in an Italian study in adults commencing PI-based HAART[420] Indeed, estimates of specific body fat alterations were similar to those reported in the LipoICoNa study of 655 adults where incident lipohypertrophy was reported as 5.5 per 100 person-years, lipoatrophy as 3.8 per 100 person-years, and the combined lipohypertrophy with lipoatrophy had an incidence of 2.6 per 100 person-years[421]. Only one previous study reported incidence of body fat alterations: in a cohort of 48 HIV-infected children (aged 6-15 years) living in the USA, the incidence was reported as being 31% after follow-up of 18 months[204]. However, estimates of incidence were lower in a African study of HIV-infected adults[400]. While the current study reported similar estimates for incidence of lipoatrophy and lipohypertrophy, two North American studies in HIV-infected adults have reported the incidence of lipohypertrophy being smaller than that of lipoatrophy[406,422,423],

Previous adult studies have reported the incidence of hypertriglyceridemia to be higher than the incidence of hypercholesterolemia in the same study population[410]. However, in common with the current study, the comparatively higher incidence of hypercholesterolemia in HIV-infected adults has also been reported[241,406]. The incidence of metabolic abnormality in this investigation was 4.8 per 100 person-years, while the incidence of hypercholesterolemia was 5.6 per 100 person-years. This hypercholesterolemia incidence is higher than that reported in the PACTG 219C study in HIV-infected children: 3.4 cases per 100 person-years (95% CI: 3.0, 3.9)[197]. This difference may be partly attributable to differences in diagnosis in hypercholesterolemia between the two studies, and a lower mean age (9.2 years) within PACTG. Furthermore, a greater proportion within PACTG was of non-White ethnicity (86% vs. 30.4% in the current study): HDL-cholesterol levels have been shown to be higher in children and adolescents of Black compared to White ethnicity[424-426]. In comparison with adult studies, incidence of metabolic abnormality in this study was lower than the incidence to the Metabolic Syndrome (defined as ≥3 of hypertriglyceridemia, low HDL-cholesterol, hypertension, abdominal obesity and high serum glucose) in a US HIV cohort[408], but higher than the incidence of severe hypertriglyceridemia (serum triglyceride >531mg/dL) in a French cohort[409].

8.2.2 Factors associated with lipodystrophy syndrome

Protease inhibitors

As discussed in Chapter 1, several mechanisms of PI-induced pathogenesis of LS have been proposed. These have included direct effects on glucose production [255,256], glucose disposal[257], triglyceride metabolism[263,427], and adipocyte differentiation and apoptosis[221,255,265-267.] At recruitment, 56% of subjects were being treated with PIs: use of PI at recruitment was significantly associated with increased risk of concurrent lipoatrophy and lipodystrophy (AOR: 5.81) in cross-sectional analyses. However no individual PI (including ritonavir booster) remained significant in any multivariable model: this may be because there was insufficient statistical power to detect an association in the multivariable models as there was no significant difference ($p \geq 0.341$) in the proportions of subjects with body fat alterations either on or not on PIs at recruitment. However, significant ($p < 0.01$) differences were seen between the proportions of subjects who had ever been on PIs with respect to all body fat alterations outcomes. This may indicate that body fat alterations had occurred in these subjects, and they had been switched to less toxic medication before enrolment making it more difficult to identify an association between current PI and body fat alteration at recruitment. The associations between PIs and LS are well recognised, with prescribing information for many PIs explicitly stating that adverse reactions for these drugs include LS. In adult studies PI use has been shown to be associated with lipohypertrophy, but inconsistently with lipoatrophy[184,214,226 215].

An increased risk of body fat alterations with PI-use (AOR: 7.79) was seen in 88 HIV-infected children (mean age: 11.1 years) living in Belgium in analysis adjusted for gender, age, CDC stage, CD4 counts and duration of treatment[205]. Furthermore, the European Paediatric Lipodystrophy Group reported a significant increased risk of any body fat alterations associated with ever use of PIs (AOR: 2.41, 95% CI: 1.18, 4.91), in multivariable models of 423 children aged >3 years adjusted for sex, age, CDC clinical class, and ever use of stavudine[209]: no significant association was seen with either central lipohypertrophy or peripheral lipoatrophy. However the evidence of an association has been weaker or absent in two US-based paediatric studies: PI treatment was associated with a significant increased risk of body fat alterations (OR: 7.0, 95% CI: 1.1, 45.2) in unadjusted analysis of pre-pubescent children (mean age: 7.5 years) in the first[200], and no significant association between duration of PI use in children and body fat alterations was seen in the second study[195]. However both these studies were small (40 and 28 subjects respectively) cross sectional studies.

In this study, use of PI at recruitment was associated with a significant 2-7 fold increased risk of all metabolic abnormality outcomes in multivariable logistic regression models including class of ART, while recruitment ritonavir was associated with a significant 2-8 fold increased risk in additional models: in both sets of models the greatest magnitude of risk was seen for concurrent hypercholesterolemia and fasting hypertriglyceridemia. Furthermore, use of PI was associated

with a 4-4.5 fold increased risk of incident metabolic abnormality (Chapter 6). Adult studies have shown elevated triglycerides and total cholesterol levels to be associated with any PI[184,186,216,226,397,428], and with ritonavir in both HIV-infected[262,396,402,429,430], and uninfected subjects[431]. Paediatric studies have also demonstrated PI use to be associated with hyperlipidemia[213,360]. In a Swiss study of 66 children aged <17 years, PI-use was significantly associated with increases in both total cholesterol and triglyceride levels in previously PI-naïve subjects, with the greater increases seen with ritonavir-treated compared with nelfinavir-treated children: ritonavir use was associated with a non-significant 70% increase in risk of hypercholesterolemia (defined as ≥90th percentile for age and sex) compared to nelfinavir use (AOR: 1.7, 95% CI: 0.4, 6.6)[432]. Two small prospective studies with subjects aged <18 years switched from PI-based cART to NRTI/NNRTI-based regimens reported decreases in serum levels of total cholesterol and triglyceride after 48 weeks of follow-up[246,433]. Respective estimates for the independent risk associated with PIs for dyslipidemia, hypercholesterolemia and hypertriglyceridemia in children are 2-fold[211] (multicentre French study of 130 subjects), 5-14 fold[196,197] (≤2122 subjects enrolled in the PACTG 219C), and 3-fold (178 subjects enrolled in the Perinatal AIDS Collaborative Transmission Study, PACTS, and 178 children enrolled in a single-centre US study)[203,359].In addition to inherent differences in the study population and factors in the statistical modelling, the heterogeneity in the definitions of metabolic abnormality[j] may have contributed to the range in estimates of the effect size. However, the estimates from previous paediatric studies are in broad agreement to those calculated here: within the PACTS-HOPE study, PI-inclusive regimens were independently and significantly associated with a 4-fold increased risk of cholesterol ≥200mg/dL, and a 2-3 fold increased risk of hypertriglyceridemia. Furthermore, on restricting the study population to subjects who were PI-treated in PACTS-HOPE, a 2-fold increased risk of hypertriglyceridemia was seen with ritonavir containing regimens (AOR: 2.1, 95% 1.2, 3.5)[203]. Comparison of the magnitude of the independent associations between paediatric and adult studies suggests that it is possible that children and adolescents are at greater risk of developing LS than adults.

The association of PI use with a poor metabolic profile is underscored by the results from the multivariable multilevel model: recruitment PI use was associated with significant increases in total cholesterol, LDL-cholesterol, non-HDL cholesterol and fasting triglyceride, with time-updated PI use associated with increases in both LDL-cholesterol and non-HDL cholesterol. Although evidence from the current study strongly suggests that PI use is a risk factor, there is a possibility of residual confounding and confounding by indication. Moreover, the follow-up time may be too limited to investigate the effects of these newer drugs.

[j] The French study defined elevated triglyceride and cholesterol as >+2 standard deviations in *z*-scores from age/gender/ethnicity defined reference distributions, the P219C study defined hypercholesterolemia as serum cholesterol ≥220 mg/dL, PACTS-HOPE defined hypertriglyceridemia as triglycerides ≥150mg/dL and hypercholesterolemia as cholesterol ≥200mg/dL, while the US study defined hypercholesterolemia as cholesterol ≥200mg/dL.

Non-nucleoside reverse transcriptase inhibitors

At recruitment one third of subjects were being treated with NNRTI. Use of NNRTI was a significant risk factor for any fat alterations, lipohypertrophy, lipoatrophy, and concurrent lipohypertrophy with lipoatrophy. In analyses incorporating specific drugs, efavirenz but not nevirapine was associated with a significant increased risk of any body fat alterations. Few paediatric studies have found an association between use of NNRTI and body fat alterations: no significant association has been reported in a US-based investigation, but this study only included 7 subjects with body fat alterations[195]. However, several investigations in adult populations have reported a significant association. Analysis of 338 women enrolled in FRAM identified NNRTI to be a significant risk factor for lipoatrophy in the leg in adjusted analyses[434], while multivariable analysis of 416 adults in an American cross-sectional study found efavirenz and nevirapine to be significant risk factors for dorsal lipohypertrophy[236]. Furthermore, the DAD study showed that NNRTI-use was significantly associated with a 13-fold (AOR: 12.99, 95% CI: 9.46, 17.8) increased risk of body fat alterations[394]. Although results from the current study are consistent with adult studies suggesting that NNRTI, and specifically efavirenz, is a risk factor for body alterations, there is an absence of (both paediatric and adult) studies which have investigated comparisons of risk for different fat alteration outcomes. Thus, the finding that the risk associated with concurrent lipoatrophy and lipohypertrophy was approximately 4 times that associated with other body fat outcomes may be novel.

Within this study, time-updated NNRTI use was significantly associated with an increased total cholesterol concentration in multivariable multilevel models, most likely driven by significant increases in HDL-cholesterol, as NNRTI use was not significantly associated with changes in either LDL-cholesterol or non-HDL cholesterol. In a recently published prospective study of HIV-infected children, Jacobson and colleagues, also used multivariable multilevel modelling to investigate cholesterol levels[361]: total cholesterol, LDL-cholesterol, non-HDL cholesterol and, interestingly, HDL-cholesterol levels all increased significantly with duration of use of nevirapine, efavirenz, nelfinavir, and lopinavir in 449 children followed for a median 4.5 years. These results support the findings here that both NNRTI and PI are significantly associated with increased total cholesterol, despite the age of participants being substantially lower (6.6 years at baseline).

Results from the current study suggest that NNRTI use may be associated with an anti-atherosclerotic cholesterol profile in children and adolescents, consistent with previous studies where NNRTI-based regimens were associated with increases in HDL-cholesterol and decreases in the total cholesterol to HDL-cholesterol ratio[435-438]. Moreover, in a randomised controlled trial of HIV-infected adults where PI in ART regimens were substituted, replacement

with NNRTI was associated with significant increased HDL-cholesterol and significant decreased non-HDL cholesterol[439].

In multivariable multilevel models here, NNRTI during follow-up was associated with increased fasting triglyceride. This may seem a puzzling result as several studies have demonstrated a more healthy metabolic profile associated with NNRTI-based ART in comparison to PI-based ART[435,440-442]. Jacobson and colleagues reported mixed results: 2-3 years of nevirapine use was associated with significant decreases in triglyceride levels, while <2 years and >3 years was non-significant, and >2 years of efavirenz use was associated with significant increases[361]. Indeed previous studies in adults have reported a better triglyceride profile to be associated with nevirapine compared to efavirenz[443-445]. However, it is possible that the result in the current investigation is an example of confounding by indication where children have developed a poor metabolic profile due to long-term exposure to PIs, and thus been switched to NNRTI as a management strategy of LS symptoms. This argument is strengthened by the fact that recruitment NNRTI did not remain significant in the final models for triglyceride levels. Taken together, results from this study suggest that NNRTIs are associated with body fat alterations, and also a healthier metabolic profile (through increased HDL-cholesterol). It is possible that these results may have been confounded by switching to NNRTI in order to either improve metabolic profile in children with LS, and thus an erroneous association with body fat alterations has been made. However, given that both improved metabolic profile and increased body fat alterations have been shown in other studies, the possibility of this confounding by indication recedes. The clinical importance of the simultaneous pro-body fat alterations and pro-HDL cholesterol of NNRTI is substantial. Patients on these drugs would require to be routinely, and possibly frequently, examined for both aspects of LS, especially in cases where a child with body fat alterations may switched to NNRTIs to improve metabolic profile.

NNRTIs are mildly inhibitive of the protein SREBP[446] (Chapter 1), which can lead to increases in triglyceride and cholesterol concentrations, and increased peripheral lipoatrophy, as seen in PIs (where the inhibition is stronger)[286]. This mechanism supports the postulated role of NNRTI in body fat alterations, and may partly explain a possible role in increasing HDL-cholesterol. The observation that NNRTI inhibition of SREBP is to a lesser extent than that seen with PI induced inhibition[446] may support association of NNRTI with increased HDL-cholesterol, but not with non-HDL cholesterol seen in the current study.

Nucleoside Reverse Transcriptase Inhibitors

Several mechanisms of NRTI induced body fat alterations have been proposed, relating to the postulated mitochondrial toxicity of these drugs (Chapter 1)[273,277,278,283]: stavudine is well recognised for its association to LS in adults and children, particularly with lipoatrophy[215,399,447].

The European Paediatric Lipodystrophy group reported increased risk of body fat alteration associated with stavudine – of around 3-fold for any alterations and for lipohypertrophy alone, and 4-fold for lipoatrophy[209], while in an analysis of 88 children resident in Belgium, use of stavudine was associated with a significant and independent 14-15 fold increased risk of body fat alterations[205,238]. The finding here that stavudine use at recruitment was significantly associated with increased risk of any body fat alterations, and lipoatrophy was therefore expected. Given current guidelines recommending avoidance of this drug if possible, it was reassuring to see that only 11.2% of subjects were on stavudine at recruitment, with 51.3% having used the drug in the past.

Several prospective studies in adult populations have identified either NRTI or specifically stavudine as risk factors for body fat alterations (including the specific phenotypes of lipoatrophy, lipohypertrophy and the concurrent phenotype)[199,235 404,406,421]. In prospective trials involving HIV-infected adults with lipoatrophy, switching from NRTI-based therapy to abacavir or tenofovir was shown to be significantly associated with reversal of fat alteration symptoms[237]., Indeed, within this study, ever-use of stavudine was associated with significant and independent 2-5 fold increased risk of all body fat alteration outcomes. Longer duration of NRTI use was associated with significant increased risk of lipoatrophy in adjusted analyses from the Western Australian Study HIV Cohort [235]. Furthermore, within the same study, the effect size was almost increased from 10% to approximately 200% in cART with PIs. The authors argue that PIs may be the predominant influence in lipoatrophy, and may act synergistically with NRTIs[235].

An unexpected finding in this study was the statistically significant decreased risk of concurrent lipoatrophy and lipohypertrophy associated with zidovudine, and lamivudine. Indeed, this combination of drugs has been associated with lipoatrophy[448]. It is unlikely that these associations are the result of confounding by indication. Neither lamivudine nor zidovudine has been associated with improved LS profile in other studies; furthermore, confounding by indication would give an erroneous positive association unlike the negative association observed. Median duration of ART use in subjects with concurrent lipoatrophy and lipohypertrophy and who were on either of these drugs here was 6.05 years (IQR: 1.25. 9.12), with median age of 13.15 years (IQR:10.30, 15.38): thus these subjects may be long-term non-progressors who had not switched to newer ART. Subgroup analyses suggested that the negative association observed between lamivudine and zidovudine with the combined phenotype was driven by males and children/adolescents aged > 11 years. Studies in adult populations have shown a positive association between specific body fat alterations and older age[434,447], or female gender[199,412,413]. However few studies have investigated the combined phenotype[277,278,283].

Within the current study, both PI and NRTI were significant risk factors for body fat alterations including lipoatrophy. It has been argued that because PI/NRTI-associated lipoatrophy occurs at

a faster rate to NRTI-associated lipoatrophy, then PI therapy has a strong independent effect on fat loss, and long-term NRTI therapy may lead to a predisposition to lipoatrophy[235]. In Cox adjusted analyses of 178 HIV-infected children followed up for ≥10 years, NRTI mono-therapy, NRTI/NNRTI dual therapy, and NRTI/PI dual therapy were all associated with increased risk of triglycerides >200mg/dL: this was statistically non-significant in the first group but significant in the latter two groups with the risk greater with NRTI/PI (AHR: 3.45, 95% CI: 2.65, 4.51) compared to NRTI/NNRTI (AHR: 1.86, 95% CI: 1.34, 2.19)[359]. Moreover, in subgroup analysis of 94 previously ART naïve adults in the Prometheus randomized clinical trial, patients on ritonavir/saquinavir/stavudine had an independent and significant 7-fold increased risk of body fat alterations compared to patients on ritonavir/saquinavir: this association was also significant in patients regardless of previous ART status[449]. Furthermore, adults who developed lipoatrophy in a prospective French cohort were shown to have accumulated longer durations of NRTI treatment before they commenced HAART compared to those not developing lipoatrophy[450]. Thus it is possible that the children in this cohort may not have accumulated durations of NRTI therapy associated with the increased risk seen in adult studies. Further analysis in deciphering the ART history of subjects in this study, e.g. the time on NRTI and PI therapies and the time on combined NRTI and PI therapy, may help to ascertain whether a similar synergistic effect of these drugs occurs in children with shorter durations of treatment in comparison to adults .Simplification of treatment with PI-based mono-therapy has been associated with lower viral suppression and increased risk of virological failure in adults[451]. An on-going observational study of 5 children switched to PI-based mono-therapy reported controlled viral load, increased CD4 count, no side-effects, and improved or maintained serum lipid concentration (at 20 months of follow-up): such results need to be validated in large paediatric clinical trials before their routine use as maintenance therapy can be ascertained[452].

HIV-associated clinical disease and immunosuppression

The possible role of HIV, as indicated by viral load, immuno-suppression and clinical disease in LS pathogenesis is complex. During HIV-infection, the differential pathways of immune system activation are mediated by inflammatory cytokines (the HIV-specific response) and homeostatic cytokines (the homeostatic response to lymphocyte depletion)[453-455]. Both increased cytokine secretion from adipose tissue and increased systemic pro-inflammatory cytokine activity have been speculated to have a significant role in the pathogenesis of LS[221,222,456-458]. It is possible that the HIV-induced cytokine pathways may also have a role in body fat alterations and metabolic abnormality: common cytokine pathways may be multifaceted and thus difficult to elucidate.

Over 57% of subjects enrolled in the cohort had a history of symptomatic HIV disease, including 22% with AIDS, while over 62% had moderate or severe immunosuppression at some point during their lifetime. Experience of symptomatic HIV disease was an independent risk factor in multivariable models for any body fat alterations, and lipohypertrophy: a history of AIDS was associated with 2-fold increased risk of any body fat disorder, while CDC clinical stages B and C were each associated with a 2-fold increased risk of lipohypertrophy. The effect size is comparable to those reported in the European Paediatric Lipodystrophy Group Study[209]. However, this previous study also reported a significant two-fold increased risk of lipoatrophy[209] which was not seen here. The role of HIV-associated disease as a risk factor was supported by the finding that incident LS was significantly associated with a history of moderate CDC-defined clinical.

The Italian Coordinators for the Study of Allergies and HIV Infection (CISAI) cohort of adults reported a 30% increased risk of lipoatrophy with an AIDS-defined clinical status[404], supporting the finding in cross-sectional analysis here of a 2-3 fold increased risk of body fat alterations, lipohypertrophy and LS with symptomatic clinical status. It is possible that past disease may predispose children to LS, and specifically lipoatrophy. Indeed, growth retardation in HIV-infected children compared to uninfected children is well established[230,459], indicating the direct impact on body habitus of untreated HIV.

In the longitudinal analyses of lipids here, maximum CDC-defined clinical status B was associated with increases in total cholesterol, which was driven by significant increases in LDL-cholesterol. Although no association was found with triglyceride levels, AIDS-defining illness has been associated with a significant and independent increase in triglyceride levels in HIV-infected men[460], with cytokine pathways proposed to be a potential mechanism[461]. It can also be speculated that subjects with a history of severe disease may have accumulated more complex treatment history than subjects who had been healthy. Although duration of ART included in all analyses would control for some of the confounding due to previous treatment history, further analyses of the cohort investigating specific treatment history will help to address this theory.

In cross-sectional analysis, recruitment immunosuppression was not a significant risk factor for any fat alteration outcome in the current study (106 subjects in total had moderate or severe immunosuppression at recruitment). However a reduced risk of incident body fat alterations was significantly associated with the occurrence of any immunosuppression during follow-up (AHR: 0.41, 95% CI: 0.19, 0.91). An association between immunosuppression and body fat alterations has been reported in adult studies: significant reduced risk of lipoatrophy associated with increasing current CD4 cell count (AOR: 0.58 per 100 cell increase, 95% CI: 0.36, 0.93), was found in the NOVAVIR trial[462], and an even greater reduced risk of incident cases was seen in an Italian study[407].

Observational studies in adults have also demonstrated that increased of risk body fat alterations and/or metabolic abnormality are significantly associated with reductions in both current[215] and nadir cell count[463,464]. However, in the current study, the occurrence of immunosuppression at any time during follow-up was associated with a significantly reduced risk in the incidence of metabolic abnormality (AHR: 0.34, 95% CI: 0.12, 0.98). Indeed, in multivariable multilevel models the occurrence of immunosuppression over follow-up was associated with a significant decrease in non-HDL cholesterol. This is in agreement with results from the PACTS-HOPE paediatric cohort, which demonstrated that severe immunosuppression (AOR: 0.4, 95% CI: 0.2, 0.95) and undetectable viral load (AOR: 2.5, 95% CI: 1.5, 4.1) were significant risk factors[203]. Indeed, detectable viral load was also independently and significantly associated with significant decreases in all cholesterol outcomes (7-19mg/dL) in the current study. This negative association may be representative of reduced adherence to drugs and thus a reduction in the pro-LS effects of ART.

Within the current investigation, detectable viral load was not a significant risk factor for incident metabolic abnormality, but immunosuppression during follow-up was associated with reduced incidence and PI use at recruitment with increased risk. No interaction between these latter factors was found. However, within the PACTS-HOPE study, severe immunosuppression remained a statistically significant risk factor for hypercholesterolemia, even after exclusion of subjects not on PI-based regimens[203]. Thus it is possible that reduced CD4 counts have an independent protective effect against metabolic abnormality.

This study has shown that time-updated markers of HIV during follow up were associated with reduced risk of LS outcomes in prospective analyses, whereas markers of maximum and recruitment HIV were associated with increased risk in cross-sectional analyses. This may be indicative of the pathogenesis of LS being associated with past symptomatic disease and/or complex treatment history, with reduced occurrence with incomplete adherence to ART. Furthermore, the concurrent findings in longitudinal models that detectable viral load was associated with reduced total cholesterol, and clinical symptoms with increased LDL-cholesterol, may suggest that there may be an independent protective effect with markers of HIV.

Age and Puberty

At recruitment, 10.0% of the study population were aged 2-6 years, 27.5% were 7-11 years, and 62.4% were 12-18 years. Increasing age was a risk factor for all body fat alteration outcomes in various multivariable models, with the greatest magnitude of risk seen for lipoatrophy (5-fold in models including specific ART regimen as an explanatory variable). Although significant associations between age and body fat alterations have been reported in adult studies, the

magnitude of the effect size was smaller[447]. However it is difficult to be certain to what extent observed body fat alterations in this study are the result of "normal" age-associated fat gain unrelated to HIV in adults[465-467]. Indeed, the natural development of adipose tissue in childhood may have a protective effect against LS, which wanes during and after puberty[194].

Puberty (as measured by Tanner score) was not included in the stepwise variable selection in multivariable modelling in the current study: this was to prevent over-adjustment as all models included age, and sex was included in the covariate selection process. However, puberty was then included in the final multivariable models in additional analyses in order to investigate whether there was an independent effect. Tanner score was statistically significant in univariable models for decreased risk of metabolic abnormality, with subjects who were undergoing puberty or who had completed puberty being at reduced risk of all outcomes, but did not remain significant in any multivariable model. However, independent significant 10-29% reduction in risk per year in age of all metabolic outcomes was seen. Other paediatric studies investigating hypercholesterolemia have also reported reduced risk in older children and adolescents compared to younger children. Within the PACTS-HOPE study, children aged 11-15 years (AOR: 0.4, 0.98, p = 0.045) was associated with increased risk of metabolic abnormality (cholesterol ≥200 mg/dL and triglyceride ≥150 mg/dL), compared to being aged ≤ 5 years in multivariable models[203]. In the PACTG 219C study, in comparison to adolescents aged 12-19 years, younger children had a significantly increased risk of hypercholesterolemia: 3 fold in 4-5 year olds and 2 fold in 6-11 year olds[196].

Although, both fasting and non-fasting triglyceride levels have been shown to increase with age in healthy children[468,469], there was no significant association between age and change in fasting triglyceride levels. Prospective population-based studies in non-HIV infected children and adolescents have shown that cholesterol levels increase towards puberty, followed by a fall at puberty, and finally return to pubertal levels in late adolescence[470-475]. While multilevel multivariable models showed that older age was associated with decreases in all cholesterol levels, these were relatively small and included decreases in the anti-atherosclerotic HDL-cholesterol. Older age (12-18 years) at recruitment was also associated with non-significant changes in triglyceride levels.

The reduced risk of metabolic abnormality with increasing age may be partly attributable to worse adherence to ART in adolescents, since there is some evidence to suggest that younger children show better adherence than older adolescents[149]. Within this study, age was a significant risk factor for metabolic abnormality in subjects with poor adherence (using detectable viral load as a proxy measure) and age only remained statistically significant in multivariable models restricted to subjects with undetectable viral load, whilst remaining non-significant in most models in subjects with detectable viral load. However, detectable viral load

may be a problematic proxy for adherence in this population, and further investigation is required to understand the effects of age on metabolic abnormality.

Sex

Both paediatric [209] and adult[476] studies have reported that females may be more likely to have body fat alterations, with some studies only finding an increased risk associated with lipohypertrophy[447]. Female gender was associated with a significant (unadjusted) 76% increased risk of lipohypertrophy in the current study, but no other significant associations between sex and body fat abnormality outcomes were found. When specific body sites were investigated, girls had increased probability of lipohypertrophy in the trunk or neck compared with boys.

With respect to metabolic abnormalities, female sex was associated with a non-significant reduction in risk of dyslipidemia in unadjusted analyses, and was not significant in multivariable models. This is consistent with previous prospective adult studies which have reported non-significant elevated triglyceride to be associated with male sex[407,477]. However, female sex was associated with a modest, but significant, increase in levels of HDL- cholesterol (4.94 mg/dL, 95% CI: 1.56, 8.32) in multilevel models here. Differences between cholesterol levels between males and females may be explained by the negative regulatory role of androgenic hormones, and the positive regulatory role of estradiol on apolipoprotein A1[478] which plays a critical role in removal of cholesterol from peripheral cells[479,480].

Sex hormones have been hypothesized to have specific effects on lipid levels in non-HIV-infected children. Free testosterone was associated with increased triglyceride and LDL-cholesterol, and decreased HDL-cholesterol; while increased estradiol was associated with increased HDL-cholesterol, and decreased triglyceride and LDL cholesterol in boys aged 10-15 years old[481]. Furthermore, differences seen in hormone levels between boys of Black ethnicity and White ethnicity in the same study population illustrate the more atherogenic lipid profile in White boys following puberty[482]. Longitudinal studies in non-HIV infected populations have shown that girls have higher mean cholesterol compared to boys of a similar age[418,483]. .

Pharmacokinetic differences between sexes may be implicated in pathogenesis of LS. Indeed, the greater incidence of ART-associated toxicity in females has been attributed to increased intracellular concentrations due to reduced clearance compared to males. Women on NRTIs have higher intracellular concentrations of NRTI tri-phosphates (a proxy measure of the drug) and achieve viral suppression twice as fast as men, which may explain the greater NRTI-associated toxicities seen in women[484]. Higher incidence of NNRTI-related toxicities in women have also been reported, and attributed to reduced clearance rates compared to men[485].

Furthermore, while increased intracellular concentration of PI has been identified in women compared to men, this statistically significant difference has not been seen in the absence of ritonavir boosting[486]: this may explain why associations with LS were found with ritonavir booster in this study and not for other specific PIs. Continued follow-up of the cohort will enable analyses exploring the interaction between gender and specific ART category/drug with sufficient statistical power.

Ethnicity

The majority of subjects were of White ethnicity (69.7%), with 26.2% of Black ethnicity. Results of the current investigation show White ethnicity to be associated with body fat alterations and lipoatrophy specifically. Furthermore, in the final multivariable model for concurrent lipoatrophy and lipohypertrophy using classes of ART, White ethnicity was a significant risk factor (AOR: 4.28, 95% CI: 1.09, 16.79), compared to Black ethnicity. Studies in HIV-infected adults have demonstrated White ethnicity to be an independent and significant risk factor for lipoatrophy or any body fat alterations, but not lipohypertrophy[215,403]. Previous paediatric studies have either not addressed ethnicity as a risk factor for body fat alterations, or found no significant association.

An unexpected finding in this investigation was the significant reduced risk of incident LS with White ethnicity compared to Black ethnicity (AHR: 0.15, 95% CI: 0.05, 0.38, p <0.001). In previous cross-sectional analyses, White ethnicity had been a positive risk factor for any body fat alterations, lipoatrophy and LS. Furthermore the Western Australian Cohort of HIV-infected adults also reported a significant increased risk of emerging lipoatrophy associated with White ethnicity[235]. Interaction between ethnicity and age was significantly associated with incidence of LS (AHR: 8.47. 95% CI: 1.33, 53.94), suggesting that puberty and post-pubertal development across White and Black ethnic groups may have a different impact on LS emergence.

White ethnicity was associated with a non-significant increased risk of both metabolic abnormality and fasting hypertriglyceridemia in cross-sectional multivariable logistic models in the current study. The lack of a significant association between ethnicity and metabolic outcomes in this study may be due to the incorporation of clinical site in the multivariable models: ethnicity was significantly associated with clinical site with 15% of Italian residency subjects of Black ethnicity and 63% of Belgian residency: all subjects residing in Poland were of White ethnicity. Thus the effects of ethnicity may be diminished by the random effect included for hospital of treatment.

Previous paediatric and adult[487] studies have found White ethnicity to be a significant risk factor for poor metabolic status. In the PACTG 219C White ethnicity was a significant risk factor

compared to Black ethnicity (AOR: 2.2, 95% CI: 1.4, 3.3)[196], and African ethnicity was associated with a reduced risk (AOR: 0.34, 95% CI: 0.14, 0.80) of low HDL in the French multicentre prospective study of 130 HIV-infected children[211]. Indeed, analysis of Project Heartbeat data (on non-HIV infected children) demonstrated that sex and age-dependent trajectories of total cholesterol, HDL-cholesterol, LDL-cholesterol and triglyceride differed by ethnicity, with Black children tending to have lower LDL-cholesterol and triglycerides, and higher HDL-cholesterol[488]. Furthermore, the Metabolic Syndrome has been shown to occur less frequently in individuals of Black compared with White ethnicity in non-HIV infected populations[489]. These previous studies are consistent with the finding here that White ethnicity was associated with significant decreases in HDL-cholesterol (and thus total cholesterol) compared to Black ethnicity.

White ethnicity was also associated with significant and independent increases in fasting triglyceride in multilevel models explored in the current study. Lower triglyceride levels in people of Black ethnicity compared to those of White ethnicity have been reported in healthy subjects[490], with the comparatively better lipoprotein profile in Afro-Caribbean men living in the UK attributed to secretion of large proportions of their VLDL as small, triglyceride-poor particles[491]. Thus underlying genetic differences (either independent of, or synergistic with HIV or ART pathways) are likely to effect the risk and manifestation of LS between Black and While populations.

8.2.3 Relationship between body fat alterations and metabolic abnormality

In order to establish whether metabolic abnormality precedes body fat alterations, or vice versa, a series of analyses investigating the relationship between both aspects of LS seen in this cohort were conducted: the aim of this was to gain further insight into the pathogenic process of LS.

Within this cohort, overweight BMI at any time was associated with significant increased risk of incidence of metabolic abnormality. Although a prospective adult study reported a significant positive association between BMI and lipoatrophy[400], a previous paediatric prospective paediatric study did not find an association between BMI and body fat alterations[204]. Significant increases in non-HDL cholesterol, and significant decreases in HDL cholesterol have been seen with increased BMI in both non-HIV infected children[492] Association have also been reported between adiposity and other biomarkers (increased blood pressure, apolipoprotein B, C-reactive protein and IL-6, and decreased apolipoprotein A), suggesting that adiposity may foster an adverse cardiovascular and metabolic profile from early childhood[492]. However, BMI may be an inappropriate measure of body fat alterations in children. For example a subject who develops both lipoatrophy and lipohypertrophy may have similar BMI both before and after the

development of body fat alterations. Furthermore, either lipoatrophy occurring both in the trunk and the legs, or lipohypertrophy occurring in both these locations, would give a similar waist/hip ratio to a subject with no body fat alterations in these locations. Indeed, it has been argued that it may be a problematic to use BMI to identify overweight and obese children, given normal body development and localized fat accumulation[493,494].

Subjects here with incident body fat alterations had higher levels of fasting triglyceride, total cholesterol, LDL-cholesterol, and non-HDL cholesterol at the end of follow-up compared to recruitment, but this did not reach statistical significance. Furthermore, metabolic abnormality at recruitment was associated with a statistically significant 5-fold increased risk for incident body fat alterations. However, body fat alterations were not a significant risk factor for incident metabolic abnormality. These results indicate that while there are alterations in body habitus and metabolic profile that occur concurrently, there is evidence to suggest that metabolic abnormality does precede body fat alterations. Indeed, the observation that metabolic abnormality at recruitment was a statistically significant risk for incident body fat alterations, but time-updated metabolic abnormality was not, indicates that any pathogenic process for metabolic changes resulting in effects on adiposity may be slow-acting.

Previous studies investigating the relationship between metabolic abnormality and body fat alterations in LS have been restricted to HIV-infected adults. Findings from the FRAM study suggest that specific metabolic abnormalities are associated with increases in specific fat alterations[460,495]: however, the cross-sectional nature of the data limit the strength of the evidence for this to be a causative association. Similarly, other studies have reported an association between body fat alterations and metabolic abnormality, but lacked a temporal component[496-498]. In a prospective study in South Africa, subjects developing body fat alterations had significantly higher levels of circulating insulin at baseline[224], indicating that metabolic disturbances precede adipose tissue changes. Furthermore, analysis of 5-year data from the French Aquitaine Cohort showed hyperlipidemia to be significantly associated with a 2-fold increase in risk for body fat alterations[241]. In a prospective study of 58 subjects, metabolic abnormality was seen to precede body fat alterations: significant increases in triglyceride, total cholesterol and both LDL and HDL cholesterol were seen at 12 months of follow-up, while significant changes in fat distribution (limb lipoatrophy) were not seen until 24 months[499]: Further evidence for metabolic abnormality preceding body fat alterations was seen in a 1-year prospective study of 31 HIV-infected adults: subjects who developed lipoatrophy had higher levels of triglyceride, and lower levels of HDL cholesterol, with decreased insulin sensitivity at recruitment compared to those who did not develop lipoatrophy[500]. Moreover, significant reductions were seen in expression of genes involved in adipocyte differentiation, lipid uptake, and local cortisol production in thigh adipocytes in lipoatrophy patients in the same study from 2 months of follow-up[500].

The evidence for a role of body fat alterations as a risk factor for the emergence of metabolic abnormality is more mixed, as few studies have had a longitudinal approach. Within the current study, no significant association was found between body fat alterations and incidence metabolic abnormality in either univariable or multivariable models. It is possible that this study was underpowered to detect a significant effect size on incident metabolic abnormality by recruitment body fat alterations.

Advances in the understanding of pro-atherosclerotic pathways of metabolic changes have been made by investigating triglyceride and cholesterol levels in relation to abdominal obesity[501,502] , subcutaneous and visceral adipose tissue[503]. Indeed, adipokines (seen to be over-secreted from abdominal adipose tissue in obesity[504]) have a role in inflammation and postulated role in the metabolic syndrome[505].

8.2.4 Progression and regression of symptoms

All 228 subjects who had LS at recruitment experienced both progression and regression of symptoms: this most commonly manifested itself as progression of metabolic abnormality accompanied by regression of body fat alterations, although regression of both body fat alterations and metabolic abnormality was also common. Among 201 subjects with body fat alterations at recruitment, almost three-quarters experienced regression of symptoms without worsening of any symptoms; the remainder experienced both progression and regression. However, among 214 subjects with metabolic abnormality at recruitment (some of whom had concurrent body alterations) the distribution of change in symptoms was more heterogeneous: approximately one-quarter experienced regression only, one-quarter experienced progression only, and approximately one-quarter experienced both.

These results demonstrate the dynamic nature of LS in children. Furthermore, they illustrate the difficulty in monitoring patients with the syndrome. Although thresholds for hyperlipidemia were based on age and sex (with cut-off points for regression and progression of symptoms set at 20% above and below the standard thresholds for hypercholesterolemia and hypertriglyceridemia), the identification of regression of body fat abnormality symptoms was dependent on clinician assessment as to the level of and change in severity of symptoms. Thus, it is possible that the changes seen in body fat severity were more arbitrary. Furthermore, it may be that the regression seen in fat alterations may be due to expected changes seen during puberty. Other studies in HIV-infected children with LS have focused on the disappearance of body fat alterations as an endpoint[506] rather than looking at graded regression of symptoms, and therefore have not looked at the dynamism of LS-symptoms.

Various methods of assessing morphological changes in HIV-infected adults have been employed[507]. Paediatric studies have assessed body fat alterations by using measures of

skinfold thickness compared to standards[508,509], or using specific anthropometric measures, such as weight and height, to compile z-scores from distributions compiled from the underlying population[510]. Population-specific standards of waist-hip ratio have been shown to be suitable for detection of increased abdominal mass by using cut-off points based on standard deviations from the mean[511]. This approach was not suitable in this study given the mixed ethnic make-up of the cohort. Adult studies have used computerized tomography (CT) scans to estimate visceral adipose tissue and total adipose tissue by examination of cross-sectional imaging[512]. Although this approach has been used to describe body fat in a case review of HIV-infected adolescents[513], it has not been used in larger paediatric studies, due to the expense and requirement for specialist interpretation. DEXA are a straightforward method of assessing adiposity over time as (both total and specific compartmental) body fat can be estimated as both weight and as a proportion of body weight[512]. Paediatric studies have used DEXA to assess adiposity over time in HIV-infected children over time, but the study size has been relatively small ($n \leq 24$)[233,514,515]. However, as with CT, issues of expense and inconvenience may be problematic in routine DEXA use. The current study was an epidemiological observational study and thus relied on routinely collected data and did not collect data from specialized testing outside routine hospital visits.

No statistically significant associations were seen between any patient characteristics and change in symptom severity of either body fat alterations or metabolic abnormality symptoms. Thus other factors, possibly occurring over follow-up may be responsible for these changes. It is also feasible that the pathogenesis of LS may be reflected in the dynamic progression and regression of symptoms. LS is a multifactorial condition with both ART and ART-independent pathways of causation and multiple metabolic and physical manifestations: thus disease processes underlying lipoatrophy and lipohypertrophy may differ and might have critical periods of development as well as latent periods. Periods of progression lipohypertrophy may be followed by regression of lipohypertrophy, but this regression may be accompanied by a spike in lipid levels: in such a situation the child would be recorded as having regression of symptoms of body fat alterations, progression of symptoms of metabolic abnormality, and *no change* in the symptoms of LS. In these circumstances it may be that the interval between monitoring specific symptoms is too long to detect multiple changes in symptom severity over the period, or that the thresholds for detection of such symptoms are not sensitive enough. The possibility that changes in body fat alterations and changes in metabolic abnormality, although associated, are occurring at different rates makes it difficult to accurately assess the change in symptoms of the syndrome over time. This has important implications in the management of LS and evaluation of effectiveness of interventions.

8.2.5 Management of lipodystrophy syndrome

This study was not designed to investigate effectiveness of management strategies, but instead allowed description of the range and combination of strategies used. The most common LS management strategy in this study was a combination of dietary and physical activity, used in a quarter of LS cases, followed by dietary intervention alone in 20% and physical activity in 12.5%. However, for over half of children with LS, no dietary, physical activity, surgical or pharmaceutical intervention to manage LS symptoms was used.

No specific guidelines exist for the identification or treatment of LS in HIV-infected children and adolescents. However, various guidelines for the management of elevated lipid levels in children in order to reduce development of CVD risk factors exist [516,517 518 519]. As cholesterol levels in early childhood are associated with levels in adolescence and adulthood[520,521], management of hyperlipidemia associated with LS in HIV-infected children may be an important strategy in reducing CVD risk later in life.

Physical activity was one of the most common management strategies in this study, reflecting the literature demonstrating that increased physical activity and reduced sedentary behaviour is associated with a healthy weight or reduced subcutaneous fat[168,169522-526]. It has also been shown that increased physical activity in leisure time among male adolescents aged 15-21 was associated with increased HDL-cholesterol in the general population[527]. Lifestyle interventions encompassing modifications to physical activity and nutrition have been shown to be successful in positively impacting on weight[528,529]: A recent Cochrane systematic review concluded that combined behavioural lifestyle interventions compared to standard care or self-help may produce a significant and clinically important reduction in overweight in children and adolescents[530]. Thus increasing physical activity and modifying diet may result in changes in body fat, but the regularity of, and degree of effort involved in, physical activity required to either prevent weight gain or lose weight may vary in different populations and at different stages of physical development[531]. Although sedentary lifestyle has been associated with the occurrence of concurrent lipoatrophy and lipohypertrophy in a cross-sectional study of 150 HIV-infected adults[532], and a case-review in HIV-infected adults with LS showed diet and exercise changes to be linked to reduced abdominal lipohypertrophy[533], the body fat alterations seen in HIV-associated LS are unlikely to be physiologically analogous to changes in body habitus seen in overweight or obese children. Thus any interventions traditionally used to combat overweight BMI in children may not be appropriate for management for fat abnormalities associated with LS. This does not exclude the possibility that interventions (including lifestyle modification) designed to combat hyperlipidemia[527] may have secondary effects on body shape.

Dietary intervention had been recommended to 31 subjects with metabolic abnormality at recruitment, of whom 70% had hypercholesterolemia and one-third hypertriglyceridemia. There is abundant evidence that modification of diet can improve lipid profile. Both adult[534] and

paediatric studies[535-537] in non-HIV infected populations have shown that reducing dietary saturated fat can result in decreases in levels of total and LDL-cholesterol, including among children with hyperlipidemia[538]. Furthermore, increased dietary fibre has been associated with decreases in non-HDL-cholesterol[539] (and smaller decreases in triglyceride[540]) in pre-pubescent children. Although it has been suggested that vitamin supplements may reduce mitochondrial toxicity, and thus have beneficial effects on HIV-associated LS, inclusion of thiamine, riboflavin, ubiquinone and acetyl-carnitine in mitochondrial support therapy has shown mixed results[281].

Only 14 children with LS received pharmacological intervention during follow-up. This may reflect both the lack of clinical thresholds to diagnose LS in HV-infected children, and the absence of specific clinical guidelines for management of LS, and of evidence-based recommendations for management of dyslipidemia in the general paediatric population[315]. However, guidelines for identifying hyperlipidemia in children as part of cardiovascular risk factors in moderate/high risk groups have been proposed[541]. It is also possible that clinicians may have refrained from treating LS symptoms in order to avoid adding to an already complex pill burden in relation to ART. Such increased burden may have knock-on effects on adherence, especially where LS management drugs are known to be associated with paediatric adherence problems themselves, e.g. fibrates[542,543]. Another possibility is the desire to avoid toxicity in relation to combining ART and LS management drugs, e.g. specific statins have been linked to liver and muscle toxicity if used in conjunction with PIs[544]

The pharmacological interventions used in this study were growth hormone, steroids, testosterone, Omega 3 fatty acid, and statins. These were most commonly used where the child had body fat alterations. Most previous studies have focused on management of metabolic symptoms. Within the PAGTG 219C study, fewer than one in ten (8.1%) of the 624 HIV-infected children subjects with hypercholesterolemia (total cholesterol >200mg/dL) received pharmacological intervention, most commonly statins[545]. Within the current cohort, the proportion of subjects with prevalent or incident hypercholesterolemia and who were treated with pharmaceutical intervention (where data was known) was similar to the PACTG study: 9.2% (n/N = 6/65). A more recent UK-based prospective study (mean follow-up: 4.5 years) estimated the number (n = 3) of patients in their cohort (n = 445) who were eligible for pharmaceutical intervention (defined as LDL-cholesterol >190mg/dL with children with no CVD risk factors, or LDL-cholesterol >160mg/dL for children with ≥2 risk factors), but did not specify what interventions were then used (if any), and their effectiveness[361].

Within this cohort, 5 subjects, all with initial body fat alterations were being treated with growth hormone. Reduced total and abdominal visceral fat has been associated with growth hormone therapy in pre-pubertal children without HIV who were deficient in the hormone[546]. Growth hormone deficiency in HIV-infected adults in the pre-HAART era was associated with loss of lean and fat mass: however, HAART treated patients with body fat abnormalities have also

been seen to have growth hormone deficiency[547]. Reduced growth hormone secretion is associated with dyslipidemia and increased glucose concentrations in HIV-infected adults[548] and adolescents[549] with abdominal lipohypertrophy. In a double-blind randomized trial of 404 HIV-infected adults, the growth hormone releasing factor tesamorelin was associated with significant decreases in visceral adipose tissue compared to placebo: this was also accompanied by improvements in body image distress in patients with abdominal lipohypertrophy[550]. Moreover, in a recent review of 10 randomized controlled trials of growth hormone treatment in patients with HIV-associated body fat alterations (n = 1511), treatment was associated significant decreased visceral adipose tissue and lean body mass compared to placebo[551]. Growth hormone has also been shown to improve weight and height in pre-pubertal HIV-infected children[552].

There were 4 subjects treated with steroids, and a further 2 treated with testosterone, within this cohort: all 6 had body fat alterations at the beginning of treatment (1 also with metabolic abnormality), but only 1 had no symptoms at the end of follow-up. Treatment with steroids may restore the balance of lipolysis and lipogenesis regulated by cortisol and dehydoepiandristerone (levels of which are seen to change in HIV-infected adults with LS[553]), resulting in normalization of body fat changes. Androgenic anabolic steroids in tandem with progressive resistance exercise and physiologic doses of testosterone improved lean body mass in adult males with HIV-wasting[554]. Indeed, steroid treatment has been shown to increase weight in HIV infected adult men and women with wasting, albeit associated with some liver toxicity[555]. A Cochrane review demonstrated an average increase in lean body mass of 1.3kg (95% CI: 0.6, 2.0), and of total body weight of 1.1kg (95% CI: 0.3, 2.0) in 13 randomized clinical trials of HIV-infected adults where anabolic steroids were used to treat weight loss[556]. However no paediatric studies have investigated the efficacy of steroid therapy for treatment of body fat alterations.

In a recent meta-analysis of 6 studies which investigated the effects of omega-3 fatty acid in HIV-infected adults, the overall reduction in weighted mean difference triglycerides following 8-16 weeks of treatment was -80.34 mg/dL (95% CI: -129.08, -31.60)[557]. The American Heart Association (AHA) recommends that at least 5% of dietary intake should be omega-6 fatty acid, as this reduces risk of coronary heart disease compared to lower intakes through reduction of cholesterol[558]. Two children, both with initial metabolic abnormality, were treated with omega-3-fatty acids in this study. Although both fibrates[167,247] and bile acid binding resins[166,167] have been shown to improve lipid profile in children[209,249], neither of these approaches were used in the management of LS in this study population. This may be due to adverse reactions reported with fibrates[542], and adherence issues with bile acid binding resins[543].

Given the association between specific ART and LS, switching regimen may sometimes be used in the management of symptoms of LS (while maintaining viral suppression). It may also be used to try to improve adherence by use of a less toxic drug. Improvements in lipoatrophy

were seen in adult patients after cessation of treatment with stavudine[184,395]. Switching from stavudine to tenofovir improved both lipoatrophy and lipid profile in a prospective study of 62 adult patients[559]. In addition to switching from stavudine-containing regimens, moving from other ART regimens has been associated with improvements in LS-associated symptoms, including switching from PI-based regimens[560,561]. Paediatric studies have not specifically investigating switching of ART regimen in relation to management of LS: the PACTG 219C study described ART switching amongst HIV-infected children with LS, but did not relate it to symptoms[545]. Both adult and paediatric studies often have limited follow-up, with heterogeneity in LS measures, and inability to account for the impact of prior therapy[562]. Within this investigation, the examination of management of LS has been limited to non-ART based interventions. Given the rich data collected on past ART use in participants, and the continued follow-up, management of LS by switching of ART regimens may be investigated in future analyses.

8.3 Strengths and weaknesses of this investigation

8.3.1 Data

This study has several advantages over previous paediatric studies which have investigated LS. As discussed in Chapter 1, these were limited by small sample size, being cross-sectional in design, or being nested within a study which was not primarily dedicated to investigate LS. In contrast, the European Paediatric Lipodystrophy Cohort is a prospective study of 426 subjects attending specialist paediatric HIV centres across 3 European countries. The data collection tools were designed with the specific aim of capturing information on metabolic abnormality and body fat alterations associated with LS, and also on potential determinants of LS. Thus this research has the advantage of using specific LS definitions, with results being generalizable to a wide range of HIV-infected children and adolescents. In contrast to many previous studies, inference between the causal relationship between a given risk factor could be made from the longitudinal design. Furthermore, because of the range of data collected, the effects of LS on lipid levels could be modelled.

Possible sources of bias

While the multicentre structure of the cohort facilitated a large sample size and improved the potential applicability of findings to a greater population, there is the possibility that there may be systematic differences between study sites. These may take the form of different populations, differing ART regimens, or strategies in managing patients as they move through adolescence (for example in the number of routine hospital visits, or responsibility in taking on HIV treatment management). In order to address the possibility of these possible systematic biases effecting analyses, random effects for hospital were included in statistical modelling (logistic regression, Cox survival regression, and multilevel modelling).

Given the wide range of metabolic changes identified during LS[187], use of questionnaires to collect data provides a convenient approach to collecting data. Serum levels of triglyceride and cholesterol are specific continuous measures which can be interpreted within a suitable framework, e.g. threshold values dependent on sex and age. However, assessment on body fat alterations by examining changing in body habitus is less objective. Several recent studies have used DEXA scanning as a way of assessing body fat density to investigate LS[563-565], although sonography has also been suggested as a useful alternative method[566]. Indeed DEXA has been seen as a gold standard in the assessment of lipoatrophy and lipohypertrophy[286]. The observational nature of this study made it impossible to collect DEXA scans for each child as these are not part of routine hospital visits. However, body fat alterations were assessed according to strictly defined criteria using a scale of severity by the subject's established clinician. Clinical knowledge of adolescent body development, the independent effects of HIV on

growth, and body habitus changes associated with LS, alongside familiarity with the patient may reduce some subjectivity in observations of body fat alterations. Indeed, significant correlation between clinician-assessed body fat alterations, and results from DEXA scan has been reported[567]. Some earlier investigations have relied on self-reported changes which have been shown to over-reporting of body shape changes[346]: similar over-reporting is likely to occur when assessments are made by the care-givers to children and adolescents. While clinician assessments may avoid such over-estimation, they do not entirely remove the problem of reduced objectivity. Indeed, treating clinicians by their very nature are not blinded to the ART history of the patient, and thus this possible source of bias in assessments of body fat alterations cannot be excluded from consideration of the results in this investigation.

Within this study, 28% of children with body fat alterations at recruitment were affected in a single body site, with a slight increase to 30% at the end of follow-up. Furthermore, 30 subjects at recruitment were defined as having body fat alterations by trunk lipohypertrophy alone, with this number rising to 33 at the end of follow-up. This may indicate that a conservative approach was taken by clinicians in assigning a child as having body fat alterations. The collection of fat alteration by severity allowed sensitivity analyses to be conducted in cross-sectional logistic modelling: these reinforced results from the original models. It is still possible that the clinician may be more inclined to categorize a “borderline” patient as having body fat alterations where (s)he knows that the subjects is being treated with drugs previously shown to have an association: such assessments may lead to over estimation of both prevalence and risk associated with specific drug use.

While previous studies investigating hyperlipidemia in HIV-infected children and adolescents have used adult thresholds in defining cases, this study has used specific age and gender defined thresholds[568]. Thus there is greater validity in the classification of subjects with metabolic abnormality: almost one in five (19.4% n/N = 43/222) of subjects with LS at recruitment were defined by either hypercholesterolemia or fasting hypertriglyceridemia (at the end of follow-up this had increased to 21.4%, n/N= 54/257). However, up to one quarter of our study population was missing fasting measures for serum total cholesterol, HDL/LDL-cholesterol, triglyceride, glucose and insulin. Missing information on fasting triglyceride may have led to inaccuracy in estimates of prevalence and incidence of metabolic abnormality, and reduce power in statistical models. Indeed, numbers of subjects missing data on non-HDL cholesterol may have led to biases on the effects of risk factors associated with this clinically important group of lipids. Imputation was used to investigate the effect of missing values of cholesterol and fasting triglyceride on the multivariable models for hypercholesterolemia and fasting hypertriglyceridemia at recruitment (Chapter 5). The imputed multivariable models estimates of AORs were similar to the values of the models without imputed values (Chapter 5:), indicate that the missing values may not be influential on the results. However, the models with imputed values for hyperlipidemia were not able to incorporate the random effect for clinical site

because this was unsupported by the statistical analysis software (STATA v11). Thus the effect of bias due to missing values of cholesterol and triglyceride cannot be excluded. However, given the relatively small numbers of missing data, and the problems associated with using imputation in conjunction with incorporation of random effects in statistical models, it was decided not to use methods to address missing values within analyses.

Within this investigation, metabolic profiles between ethnic groups have been shown to vary: hyperlipidemia thresholds for children which take into account age, sex and gender are not yet available. Given the ethnic diversity in this study population it is possible some bias may have been retained with respect to assessment of hypercholesterolemia and fasting hypertriglyceridemia.

8.3.2 Analytical Methods

Locally weighted smoothing and fractional polynomial models were used in the description of metabolic and anthropometric measures in subjects with/without LS. Outcomes, for example HDL-cholesterol, BMI etc., were modelled with age-at-recruitment as an explanatory variable: these fractional polynomial methods were used to explore the different phenotypes of LS, and were not designed to imply a causal relationship between age and these outcomes.

Logistic regression models were performed using cross-sectional data collected at recruitment to investigate postulated risk factors associated with LS outcomes. Several factors were found to have an association with LS outcomes including PI and NNRTI use. These models can be interpreted to infer a causal relationship. However, statistical significance doesn't necessarily prove a causal association. Furthermore logistic regression models do not show a temporal relationship, e.g. PI use occurred before hypercholesterolemia occurred and thus caused it to occur. A temporal component (over the duration of follow-up) was provided by Cox proportional hazards modelling where an association between a given explanatory variable with incidence of a given outcome was investigated. Results from these models added to the argument of causality between LS and identified risk factors by calculating effect size and significance on the basis that the explanatory variable occurred before the outcome. Thus, for example, the argument for a causal relationship between PI use and LS in strengthened by the positive association between PI use at recruitment and the incidence of metabolic abnormality. Furthermore, the multilevel models also used longitudinal data and provide a temporal component in their exploration of the association between explanatory variables and serum lipid levels. These models also provide additional information on the degree to which an explanatory variable affects the cholesterol and triglyceride outcomes. For example a very significant association may result in the covariate only changing lipid levels over time, with this change not having clinical implications for the patient. However, a non-significant association may result in

the covariate causing a large change in lipid levels which may have a clinical implication. In the current study, PI use was shown to have a relatively large, and possibly clinically important, effect on serum cholesterol levels.

Explanatory variables such as clinical status and degree of immunosuppression were ordinal, but did not show linear relationships in terms of risk associated with a given outcome. It has been argued that the relationship between cause and effect should be strong, in terms of magnitude of risk: a weak relationship may occur due to confounding and/or bias[569]. Explanatory variables such as stavudine, PI and White ethnicity were associated with a relatively high 4-fold increased risk of various LS outcomes, adding to the argument for a causal association.

A benefit of logistic regression, Cox proportional hazards, and multilevel modelling is that all approaches can incorporate covariates to control for the effects of confounding. All models were adjusted for known confounders including age at recruitment, duration of ART use, and clinical site (hospital). Later models, which explored relationships between specific covariates and an outcome were adjusted by covariates that had been shown to have an association with the outcome in earlier models. However, this confounding will persist in models investigating the possible causal role of ART. Specific ART drugs were explanatory variables in several statistical models in the analyses. The distribution of these drugs in the study population would not be random (independent of LS status): treatment would be dependent on several clinical factors including disease state, immunosuppression, and adverse effects not excluding LS. It is therefore possible that residual confounding between ART and outcomes may remain even after controlling for clinical factors.

In the cross-sectional investigation of factors associated with either body fat alterations or metabolic abnormality, three sets of logistic regression models were used: (i) incorporating categories of ART and other covariates chosen by stepwise selection, (ii) incorporating specific ART drugs and other covariates chosen by stepwise regression, and (iii) including ART regimen with specifically chosen covariates chosen intuitively. The first models allowed for ART categories to be investigated with maximum statistical power in order to verify the relationship between specific categories and manifestations were as seen in the published literature. Given that different mechanisms of actions for each ART have been postulated , it was important to investigate each category separately within this thesis. Indeed this was shown to be occur: NNRTI was seen to be associated with body fat alterations (Table 8-3), and PI was seen to be associated with metabolic alterations (Table 8-4). The second set of models allowed more practical inferences to be made as they allowed distinctions to be made between drugs in the same ART category. For example, ritonavir booster was seen to be a consistent risk factor for metabolic abnormality outcomes while other PI drugs were not (Table 8-4). Indeed, newewr drugs have been developed which are designed to have less LS-associated adverse effects,

e.g. the newer PI atazanavir which was shown not to be a risk factor for metabolic abnormality in this investigation. Moreover, because all but two subjects who were currently on ART at recruitment were on NRTI, the first set of models was not able to investigate any relationship between LS outcomes and NRTI. However, because not all subjects were on the same specific NRTI drugs, the second set of models was able to establish a significant relationship between stavudine and specific body fat alteration outcomes (Table 8-3). The final set of models provided further practical results given that cART is the recommended method of treatment: these models were intuitively adjusted so that each body fat alteration model was comparable, and each metabolic abnormality model was comparable. The first two sets of models were not established by stepwise covariate selection to reduce the number of assumptions made. Moreover, since comparatively few previous studies had been conducted in children, such assumptions will have been made based upon literature for adult studies that may not necessarily have be applicable to paediatric studies.

Table 8-3: Summary of multivariable logistic regression models investigating risk factors for body fat alterations at recruitment

	Stepwise variable selection		Intutively adjusted
	Specific ART drugs	**ART categories**	**ART regimen**
Body fat alterations	Efavirenz: 1.93 (1.03, 3.62)	NNRTI: 1.97 (1.11, 3.50)	
	Stavudine: 5.40 (2.15, 13.56)		
			Triple class therapy: 5.41 (1.21, 24.13)
			Age: 1.41 (1.06, 1.23)
	White ethnicity 3.32 (1.49, 7.41)	White ethnicity: 3.48 (1.59, 7.62)	White ethnicity: 3.09 (1.47, 6.48)
	Maximum clinical status C: 2.34 (1.18, 4.65)	Maximum clinical status B: 2.40 (1.17, 4.91)	
	BMI: 1.17 (1.06, 1.28)	BMI: 1.17 (1.07, 1.29)	
Lipohypertrophy		NNRTI: 1.83 (1.03, 3.26)	
			Triple class therapy: 4.06 (1.12, 14.75)
			Age: 1.08 (1.00, 1.17)
		Female sex: 1.87 (1.08, 3.22)	
	Maximum clinical status B: 2.08 (1.07, 4.05), C: 2.24 (1.07, 4.69)	Maximum clinical status C: 2.40 (1.17, 4.91)	
	BMI: 1.46 (1.30, 1.63)		
Lipoatrophy		NNRTI: 1.85 (1.01, 3.42)	
	Stavudine: 2.69 (1.12, 6.47)		
			Triple class therapy: 8.32 (1.73, 40.07)
	Age: 1.20 (1.09, 1.32)		Age: 1.16 (1.06, 1.27)
	White ethnicity: 4.01 (1.55, 10.38)	White ethnicity: 5.08 (1.95, 13.27)	White ethnicity: 4.80 (1.84, 12.54)
	Nadir immune status 0.42 (0.21, 0.83)		

	Stepwise variable selection		Intutively adjusted
	Specific ART drugs	**ART categories**	**ART regimen**
Lipohypertrophy and lipohypertrophy occurring together		NNRTI: 7.82 (1.97, 31.04)	
		PI: 5.81 (1.46, 23.20)	
			Triple class therapy: 6.30 (1.36, 29.11)
	Zidovudine: 0.33 (0.12, 0.91)		
	Lamivudine: 0.46 (0.21, 0.99)		
			Age: 1.16 (1.03, 1.30)
	Maximum duration of ART: 1.20 (1.06, 1.36)		
		White ethnicity: 4.28 (1.09, 16.79)	
	BMI: 1.20 (1.06, 1.36)		

Table 8-4: Summary of multivariable models investigating risk factors for metabolic abnormality at recruitment

	Stepwise variable selection		Intutively adjusted
	Specific ART drugs	**ART categories**	**ART regimen**
Any metabolic abnormality	Ritonavir booster: 2.79 (1.56, 4.99)	PI: 2.26 (1.25, 4.06)	
			NNRTI-based HAART (compared to PI-HAART): 0.50 (0.28, 0.92)
			NRTI monotherapy (compared to PI-HAART): 0.23 (0.06, 0.83)
	Age (per year): 0.87 (0.81, 0.94)	Age (per year): 0.87 (0.80, 0.94)	Age (per year): 0.89 (0.82, 0.95)
Fasting hypertriglyceridemia	Ritonavir booster: 3.35 (1.60, 7.04)	PI: 2.57 (1.22, 5.49)	
			NNRTI-based HAART (compared to PI-HAART): 0.37 (0.17, 0.80)
			NRTI-monotherapy: 0.21 (0.05, 0.99)
	Age (per year): 0.88 (0.80, 0.96)	Age (per year): 0.87 (0.80, 0.95)	Age (per year): 0.89 (0.82, 0.97)
Hypercholesterolemia	Ritonavir booster: 2.82 (1.36, 5.83)	PI: 2.21 (1.07, 4.56)	
	Age (per year): 0.82 (0.75, 0.90)	Age (per year): 0.82 (0.75, 090)	Age (per years): 0.81 (0.74, 0.89)
	Detectable viral load: 0.39 (0.17, 0.86)	Detectable viral load: 0.40 (0.18, 0.89)	
Both hypercholesterolmeia and fasting hypertriglyceridemia	Ritonavir booster: 7.46 (1.66, 33.57)	PI: 6.18 (1.37, 37.85)	
			NNRTI-based HAART (compared to PI-HAART): 0.20 (0.04, 0.93)
	Maximum duration of ART (per year): 1.22 (1.01, 1.48)	Maximum duration of ART (per year): 1.22 (1.01, 1.47)	Maximum duration of ART (per year): 1.22 (1.01, 1.48)
	Age (per year): 0.71 (0.59, 0.85)	Age (per year): 0.70 (0.59, 0.84)	Age (per year): 0.70 (0.59, 0.84)

Table 8-5: Summary of multivariable models investigating risk factors for lipodystrophy syndrome at recruitment

	Stepwise variable selection		Intuitively adjusted
	Specific ART drugs	**ART categories**	**ART regimen**
Lipodystrophy syndrome		PI: 2.56 (1.20, 5.45) NNRTI: 2.78 (1.26, 6.11)	
	Efavirenz: 1.93 (1.03, 3.62) Stavudine: 5.40 (2.15, 13.56)		
	White ethnicity: 3.32 (1.49, 7.41)	White ethnicity: 3.65 (2.06, 4.46)	White ethnicity: 3.03 (1.69, 5.45)
	BMI: 1.17 (1.06, 1.28)	BMI: 1.09 (1.00, 1.19)	
	Maximum clinical status C: 2.34 (1.18, 4.65)		Maximum clinical status B: 1.86 (1.06, 3.28)

Although a statistical significant association between explanatory variables and outcomes were found, it is possible that models may have been under-powered to show such an association, i.e. the role of chance cannot be discounted. Although no ART covariate was significant in multivariable Cox models for incident body fat alterations, several ART covariates were statistically significant in cross-sectional logistic regression analyses at recruitment. However, the Cox models may have been underpowered to detect a statistically significant effect size. It is possible that the significant results seen in the metabolic abnormality models may be examples of type I errors i.e. falsely statistically significant. However, the observed results from the Cox multivariable are consistent with results seen in other studies, thus diminishing (but not excluding) this possibility.

8.4 Summary and clinical implications

This thesis has provided a body of evidence encompassing an assessment of the phenotype, identification of associated risk factors and description of management strategies related to LS in HIV-infected children and adolescents. Analyses were conducted on a large prospective cohort which allowed for appropriate measures to be taken to address bias and confounding. Moreover, the multi-centre structure of the cohort, with a diverse ethnic and age make-up means that the applicability of results is wider than previous paediatric studies. . A temporal relationship has been shown between postulated risk factors and LS. Results were consistent with previous research. Furthermore, novel research was conducted: this is the first paediatric study to estimate incidence of several LS outcomes, to investigate the association between body fat alterations and metabolic abnormality in LS, and to model the longitudinal effects of LS on specific cholesterols. However, the clinical implications must be interpreted with consideration of the underlying study population. Indeed, participants have accumulated a specific ART history which will be different to subsequent cohorts of HIV infected children. As new therapy guidelines develop, and new drugs emerge, findings may not be as applicable to subsequent cohorts of children and adolescents. Although the multi-centre design of the nature has increased power of analyses and applicability of findings, patients had distinct backgrounds, for example all Poland-residents were of White ethnicity, and >20% of subjects in Italy and Belgium were of Black ethnicity. The unique nature of this cohort must be considered when considering the applicability of the results to other populations.

This research has demonstrated that the prevalence of body fat alterations in HIV-infected children may be greater than previously thought, with 61% of subjects exhibiting LS symptoms at the end of follow-up. The most common presentation of body fat alterations occurred as lipohypertrophy in the abdomen and lipoatrophy in the limbs. In this cohort of predominantly vertically-infected children, with a wide experience of ART regimens, the incidence of body fat alterations was estimated as 8.01 per 100 person years. This is similar to estimates from adult populations. This investigation has used clinical assessments of adiposity which are practical and which not only can be used in further research, but also may be useful in detecting body fat alterations in clinical practice.

Subjects with concurrent lipoatrophy and lipohypertrophy had a metabolic profile which showed aspects of characteristic of insulin resistance. All HIV-infected children treated with ART should be routinely and regularly monitored for LS: results from this thesis suggest that children with this concurrent lipoatrophy and lipohypertrophy should be subject to increased surveillance for adverse metabolic profile and be considered for immediate management for LS symptoms in order to either prevent the occurrence of a clinically poor metabolic status, or reduce the severity of symptoms when they do occur. Furthermore, this thesis has provided evidence that suggests that metabolic abnormality precedes body fat alterations: the period between

development of metabolic changes and before the emergence of body fat alterations may provide a therapeutic window during which LS management strategies can be applied to prevent lipoatrophy and/or lipohypertrophy Further studies are required to explore the temporal relationship between the two aspects of LS, and whether any such lag period represents an opportunity for management of symptoms.

Estimates of the prevalence of hypercholesterolemia and fasting hypertriglyceridemia are similar to those reported in previous paediatric studies. Incidence of both hypercholesterolemia (4.6 cases per 100 person-years) and fasting hypertriglyceridemia (1.9 cases per 100 person years) during follow-up of children and adolescents in this study was higher than reported in previous adult investigations, which may be reflective of the larger proportion of subjects who were of Black ethnicity, (Black ethnicity having been shown to have higher lipid levels in healthy populations).

This study has shown that risk factors for LS in children and adolescents are similar to previously established risk factors in adults. As in adults, PI use, NNRTI use, stavudine use, history of HIV-associated clinical disease, and White ethnicity (compared to Black ethnicity) were independent and significant risk factors for body fat alterations. A novel finding was that NNRTI use was associated with increased risk of concurrent lipoatrophy and lipohypertrophy: previous studies have not investigated risk factors for this particular presentation of LS.

As with previous adult studies, PI use was a significant and independent risk factor for metabolic abnormality. Furthermore, PIs were associated with significant increases in total cholesterol, LDL-cholesterol and fasting triglycerides, in multilevel multivariable models: long term consequence of use may be a pro-atherosclerotic metabolic profile. NNRTI were associated with an independent and significant increase in HDL-cholesterol in multi-level models, indicating that a healthy metabolic profile, not conducive to atherosclerosis may be associated with use of these drugs. All children in this study who were on NNRTI-based therapy were on either efavirenz or nevirapine with a greater proportion on the former (70% vs. 30% respectively) These results suggest that switching children from PI-based HAART to NNRTI based HAART may limit the development of metabolic abnormality, while maintaining viral control (although other clinical considerations would be important in deciding on switching therapy in an individual patient). The most common PIs used in this cohort at the time of recruitment were ritonavir booster and nelfinavir. It is possible that future paediatric studies where participants have been exposed to newer PIs may have a better metabolic profile negating the need for switching. Indeed, given NNRTI was also associated with increased body fat alterations, the decision to switch is less clear. At the very least, these results underscore the importance of regular surveillance of body fat alterations and metabolic abnormality in tandem as different ART regimens can have opposing effects on both these aspects of LS.

Incidence of body fat alterations in this cohort was similar to that seen in previous adult studies, while the incidence of metabolic abnormality here was slightly higher: there is a need this to be validated in further paediatric studies. However, these results are reassuring in that they suggest that children and adolescents may not be at increased risk of developing LS compared to adults. However, the vast majority of children here have been vertically infected, and accumulated long durations of ART use. Adults in the previous studies will have accumulated durations of ART since, or at some point after, infection. Thus it is difficult to make a definitive conclusion about the emergence of LS in children and adolescents compared to adults. However, prospective analyses in this investigation have shown that ART is a risk factor for LS, and, crucially, is associated with increases in lipid levels in models containing LS as a covariate. Duration of follow-up was 4 years, but these results still add to the evidence of long-term toxicity of ART. Moreover, as treatment in life-long in HIV-infected children, this thesis reinforces the need to develop new therapies which address toxicities which develop over time. Moreover, studies with longer follow-up are required to address whether switching of ART (occurring as a result of treatment failure, short or long term toxicities, adherence problems, etc.) may itself have an effect on long-term toxicity.

History of CDC clinical stage B disease was significantly associated with increases in total cholesterol, LDL-cholesterol, and fasting triglyceride. Furthermore, previous serious clinical condition was also, in common with previous paediatric and adult studies, a significant and independent risk factor for lipohypertrophy. It is possible that symptomatic HIV disease history may also act as a proxy measure for complex ART history as its occurrence may have resulted in changes in ART regimen. It is therefore feasible that previous HIV-related clinical symptoms may either directly (through pathogenic processes related to HIV disease) or indirectly (through ART) lead to LS. This further underscores the need for prospective studies to investigate the association between switching therapies and long-term toxicities in children and adolescents.

Female sex was significantly associated with increased HDL-cholesterol level in multivariable multilevel models, whilst White ethnicity was associated with an independent and significant decrease in HDL-cholesterol levels, and increase in fasting triglyceride. Thus White male children may be a group at increased risk of developing LS, and perhaps be identified as a group which warrants increased surveillance when treated with ART.

The dynamic nature of LS was demonstrated by the observation that all subjects with the syndrome at recruitment experienced progression and/or regression of symptoms over follow-up. However, investigation of change in severity of symptoms highlighted the difficulties in assessing changes in degree of fat alterations during adolescence. While CT and DEXA scanning may provide more accurate determination of changes in body fat alterations, such approaches are expensive and unfeasible for assessment in routine hospital appointments.

At the end of follow-up, over 40% of subjects with LS (and where LS-management data was available) had received an intervention to treat their symptoms Dietary and physical activity lifestyle modification was the most common strategy used for management of LS-related symptoms during follow-up of the cohort. However, information on the specific approaches of implementation was not collected. Several studies have demonstrated benefits in reducing cholesterol associated with changes in diet in non-HIV infected adults and children, and thus dietary modification may be a suitable approach in the management of LS-related hypercholesterolemia. Although several physical activity interventions have resulted in weight loss in obese and overweight children and adults, it is unknown whether such approaches are applicable to HIV-infected children with LS, given that body fat alterations are likely to have a unique pathogenesis.

Numbers of subjects who were recommended a supplement or pharmaceutical intervention in the study were very small. This may be because of a lack of guidelines outlining (i) recognition of symptoms of LS, (ii) recommendations on when to commence treatment, and (iii) options for treatment. It was likely (without specific indicators for treatment data) that statins were most commonly used to manage cholesterol levels within this study population. However, statin use for the management of cholesterol in HIV-infected children must be considered carefully because of the possible adverse effects seen with PIs.

Significant associations of cardiovascular disease with both increased LDL-cholesterol, and decreased HDL-cholesterol are well established in the general population[570], with clear pathways of cholesterol metabolism to atherosclerotic plaque formation proposed[571]. The direct or indirect effect of triglycerides in the pathogenesis of heart disease is a matter of debate[572,573]. Both having HIV and having elevated triglycerides have been shown to be significantly associated with increased carotid intima media thickness in adolescents[574], with an association between elevated triglycerides and myocardial infarction (following adjustment for total and HDL-cholesterol) being shown in adult studies [575]l[576]. Hypertriglyceridemia has been associated with an independent and significant increased risk of HIV sensory neuropathy in adults[577]. Thus the clinical importance of understanding factors affecting cholesterol and triglyceride levels in children with HIV remains an important issue. Furthermore, both hypertension[578] and psychological well-being[579] in HIV-infected individuals may be associated with lipoatrophy and lipohypertrophy, underscoring the importance of understanding factors associated with body fat alterations. Thus LS remains an important issue in public health in relation to HIV, particularly in ART-treated children who, as treatment is life-long, accumulate longer durations of exposure to multiple drugs as they survive into adolescence, young adulthood and beyond.

8.5 Future work

While this study has provided compelling evidence for risk factors for LS in children, caveats on this must be applied since some of the analyses may have been statistically under-powered. Continuing prospective surveillance of this cohort will allow future analyses to confirm findings in this thesis with added statistical power. Continued follow-up of this cohort would also allow any association between newer ART drugs and LS to be investigated. Furthermore, the results of this investigation should be replicated in other studies: given the increasing availability of ART to populations outside Europe, there is a need to conduct this kind of investigation in African and Asian populations.

Changes in lipid levels over time were modelled in this study, but further analyses on the effect of body fat alterations on anthropometric measures were beyond the scope of this thesis. Future analyses should encompass modelling anthropometric measures, such as BMI and waist/hip ratio, by LS status: this may help to establish an additional framework for recognising LS in the context of routine measures. In a prospective 48 week observational study of 97 pre-pubertal (aged between 1 month and 13 years) HIV-infected children, both anthropometric and skinfold thickness measures were collected, with mid-arm and thigh muscle circumferences used to calculate lean body mass, and several of these measures used to calculate free fat mass using standard equations. Commencing or switching ART was associated with improved growth and lean body mass, and with levels of immunosuppression over time, and furthermore central adiposity was associated with detectable viral load but not ART[510]. In a more recent cross-sectional study of 369 children, DEXA scans were used to compare percentage body fat between HIV-infected children and HIV-exposed but uninfected children: specific ART and stunting was associated with specific body fat alterations[580]. Future studies may use similar assessments of lipoatrophy and lipohypertrophy to routinely identify body fat alterations, but these assessments can be validated by using DEXA scans on a sub-population of the study (thus reducing the complexity and expense of using these methods on the whole study population). Furthermore, the association between postulated risk factor and specific body fat alterations can be investigated: such studies would be important in deciphering the pathogenesis of LS if concurrent metabolic measures are taken.

As the cohort ages, new opportunities will arise to investigate social risk factors for CVD (e.g. smoking status, alcohol intake, etc.) and their role in metabolic and body fat changes. Furthermore, as participants progress through adolescence and enter adulthood, the relationship between LS-status in childhood and LS-status in adulthood can be investigated. Moreover, as all subjects reach adulthood, a complete record of anthropometric and metabolic trajectories before, during, and following puberty will be available for a substantial proportion of this cohort: a thorough investigation of LS during puberty can then be conducted.

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A.2 Abstracts arising from this thesis accepted to conferences

A.2.1 18th International AIDS Conference 2010

Risk factors for metabolic abnormality in a European cohort of HIV-infected children and adolescents

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Background
Antiretroviral therapy (ART) in HIV-infected children is associated with metabolic complications including dyslipidemia and disturbed glucose homeostasis.

Methods
HIV-infected children and adolescents were recruited to an active surveillance cohort from 14 sites in Italy, Belgium and Poland. We present baseline data on prevalence of and risk factors for metabolic abnormality (MA), defined as ≥1 of: hypercholesterolemia (HC), hypertriglyceridemia (HT), both identified according to gender/age-determined thresholds, and impaired glucose tolerance.

Results
Among 468 participants (51% female), median age was 13.5 years (IQR 9.9-17.0) with 129 (28%) at Tanner puberty stage (TAN) I and 160 (34%) at stage V. Most subjects (320, 68%) were white and 104 (22%) were of black African origin. Most (445, 95%) had ART for a median 8.8 years, with 259 (57%) currently taking protease inhibitors (PI). Fat redistribution was present in 201 children (43%). MA was present in 129 (28%) subjects: 21 (5%) had HC-only, 75 (16%) had HT-only, 4 had glucose intolerance (GI) only, 28 (6%) had both HC and HT, and one had combined GI, HC and HT. In unadjusted analyses, the following factors were associated with these outcomes: current PI use (MA, HC, HT), white ethnicity (MA, HT), advanced CDC clinical stage (MA), current NNRTI use (MA), past severe immunosuppression (HC), current undetectable viral load (<200 copies/ml) (HC), female gender (HT), TAN (HT) and country of residence (MA, HT, HC). In adjusted analyses, current PI use was significantly associated with MA, HC and HT (respective AORs [95% confidence intervals], 4.41 [2.55-7.63], 2.17 [1.03-4.59], 5.71 [3.16-10.03]); detectable VL (AOR=0.16) remained associated with reduced likelihood of HC (p<0.05)

Discussion
Over one quarter of subjects had MA, with HT the most common manifestation. Current PI use was consistently associated with increased risk of MA. The reduced risk of HC associated with detectable VL probably reflects poorer adherence to ART.

A.2.2 54th Society of Social Medicine Scientific Conference 2010

PATTERNS OF ANTIRETROVIRAL THERAPY IN A EUROPEAN STUDY OF HIV-INFECTED CHILDREN AND ADOLESCENTS

Background
Antiretroviral therapy (ART) has resulted in increasing median survival times in HIV-infected individuals by sustaining viral load suppression. Since children and adolescents are likely to have long-term exposure to ART, it is important to understand patterns of drug use to investigate the emergence of unintended sequelae.

Aim
To investigate patterns of ART in children and adolescents

Study design
Cross sectional analysis of HIV-infected subjects aged 2-22 years across 15 clinical sites in Belgium, Italy and Poland.

Method
Prevalence of both "ever-use" and "current-use" (at recruitment) of ART drug classes: Nucleoside Analogue Reverse Transcriptase Inhibitors (NRTIs); Non-Nucleoside Analogue Reverse Transcriptase Inhibitors (NNRTIs) and; Protease Inhibitors (PIs), and individual drugs were investigated.

Results
Among 468 participants (51% female) the median age was 13.5 years (IQR 9.9-17.0) with 320(68%) of white ethnicity and 104(22%) Black African. Overall, 291(62%) were virologically suppressed (HIV-RNA ≤50copies/ml) at enrolment and 35(7%) had severe immuno-suppression (age-stratified CD4%).

Only 25(5%) subjects were ART-naïve (median age=10.0 years, IQR; 3.6-11.0); 16 had experienced severe immuno-suppression or severe HIV-disease, with all having detectable viral load at recruitment.

Of the ever-treated subjects, >98% (n=436) had received zidovudine and lamivudine in the past and 74%(n=350) currently; 82%(n=368) and 72%(n=323) had been ever-exposed to PIs and NNRTIs respectively. Median and modal lifetime number of drugs was 6. Over their lifetime, 128 (27%) subjects had been exposed to ≥8 drugs (median age= 5.7 years, IQR; 12.3-18.0). Age was associated with duration of total drug use ($p<0.001$), with median age of ART initiation of 3.6 years (IQR: 1.0-7.3) Median total duration of drug use was 8.7 years (IQR= 5.7-11 years)

Nine percent of currently-treated subjects (n=38) had suboptimal management, defined as NRTIs-only (24 showing evidence of incomplete viral suppression), and 89% (n=379) had treatment with combination ART (cART).The most common PI in current-use was kaletra (n=170, 40%), possibly reflecting its availability as the only combination PI accessible in tablet/liquid form

Current-use of PIs was associated ($p<0.001$) with hypercholesterolemia in adjusted analyses indicating a long-term consequence of specific ART

Conclusions
The majority of subjects had been managed with ART, with first exposure occurring at an early age. At least a quarter of participants had been treated with multiple individual drugs suggesting cumulative exposure and switching between regimens. The most prevalent treatment approach at recruitment was cART. However, there is evidence of continued use of suboptimal management strategies, and hypercholesterolemia being associated with PI use.

A.2.3 11th Annual Workshop on Adverse Drug Reactions & Co-morbidities

Risk factors for body fat redistribution in a European cohort of HIV-infected children and adolescents

NM Alam, M Cortina-Borja, T Goetghebuer, A Vigano and C. Thorne for the European Paediatric HIV and Lipodystrophy Study

Objective
Lipodystrophy is still poorly understood in HIV-infected children and adolescents. In order to characterize lipodystrophy in this population, we established an active surveillance cohort in three European countries (Belgium, Poland and Italy).

Methods
Participants (minimum age three years) were recruited over a three month period across 14 paediatric sites by their clinicians who completed a screening questionnaire. Data collected included socio-demographic characteristics, clinical status, blood chemistry, anthropometric measures, treatment and family history. Standardized assessments were performed to assess fat redistribution in specific body areas (face, limbs, buttocks, breasts, neck and trunk). First round surveillance results are presented here. Logistic regression models were fitted to test associations between variables and different fat redistribution outcomes.

Results
Among 473 participants enrolled, 425 (89.9%) were vertically infected, and 240 (50.7%) were female. Median age was 13.5 years (IQR 9.9-17.0) with 131 (27.7%) at Tanner puberty (TAN) stage I and 161 (34.0%) at stage V. Most subjects (322, 68.1%) were white and 106 (22.4) were of black African origin. Most (419, 88.6%) had received antiretroviral therapy (ART) with a median duration of 8.8 years. Median CD4% was 31.4% (IQR 24-38.2) and 303 (64.1%) were currently asymptomatic.

At least one clinically determined sign of fat redistribution was present in 200 children (43.2%, 95%CI; 38.7-47.7): 64 (13.5%) had lipohypertrophy alone, 70 (15%) lipoatrophy alone and 66 (14%) the combined sub-type. Lipohypertrophy occurred most frequently in the trunk, with 14 cases classed as severe. Severe lipoatrophy was more frequently reported in the face. Eleven cases had both severe lipoatrophy with severe lipohypertrophy.

In univariate analyses, factors significantly associated with increased risk of any fat redistribution included white ethnicity (OR=3.31), severe HIV disease (ever) (OR 4.46 vs. never symptomatic), TAN stage V (OR 3.10 vs. TAN stage 1), maternal lipodystrophy (OR=3.15) and any exposure to “D” drugs (OR 3.73), to indinavir (OR=3.50) or to efavirenz (OR=2.62). These factors were additionally significantly associated with lipoatrophy, lipohypertrophy, lipoatrophy-without-lipohypertrophy and lipoatrophy-with-lipohypertrophy individually. Female gender was the only factor significantly associated with lipohypertrophy-without-lipoatrophy (OR=2.12).

After controlling for total duration of ART, ethnicity, stavudine, efavirenz, indinavir and the “D” drugs remained significantly associated with increased risk of all the outcomes listed above. Furthermore, white ethnicity (AOR=3.11) and use of stavudine (AOR=4.37), efavirenz (AOR=2.80), indinavir (AOR=3.23) and the “D” drugs (AOR=3.47) remained significantly associated with increased risk of the combined sub-type after adjusting for treatment duration.

Conclusion
Body fat changes were prevalent in almost 50% of this cohort, which had accumulated relatively long durations of treatment. Increased risk of fat redistribution was associated with specific drugs as well as clinical and other variables. On-going follow-up will allow further description of the lipodystrophy phenotype and investigation of its emergence, progression and management.

A.2.4 16th Conference on Retroviruses and Opportunistic Infections

Active surveillance of body fat changes and metabolic abnormalities in HIV-infected children and adolescents in Europe: first round results

Claire Thorne, Tessa Goetghebuer, Alessandra Vigano for the European Paediatric HIV and Lipodystrophy Study Group

Background: Lipodystrophy syndrome (LS) was first described in HIV-infected children nearly 10 years ago. As emergence, evolution and management of LS in children is still not clearly understood, we have established an active surveillance cohort study in three European countries (Italy, Belgium and Poland) to explore these issues.

Methods: In the initial surveillance round, clinicians from the 14 participating sites completed a screening questionnaire for all HIV-infected children/adolescents in their care over a 3-4 month period. The point prevalence of fat redistribution and dyslipidemia is estimated. Follow up evaluation will take place every 6 months.

Results: Among 435 children/adolescents enrolled, 403 (93%) were vertically infected, 226 were female, median age was 13.6 years (IQR 9.8-17.3); 30% were at Tanner stage I and 38% at stage V; 339 (78%) were white and 65 (15%) were of black African origin. 32 (7.5%) were HCV-coinfected. Most (412, 95%) were currently on HAART, 270 (62%) had viral suppression; median CD4% was 33% (IQR 25-39) and 69% were currently asymptomatic. A total of 203 (46.7%, 95%CI 41.9-51.5) children had ≥1 clinically determined sign of fat redistribution (graded as mild, moderate or severe): 64 (15%) had fat accumulation alone, mostly in the trunk with 19 presenting with severe fat accumulation; 71 (16%) had lipoatrophy alone, mostly in the legs, with severe lipoatrophy in 26, mostly in the face. Sixty-eight children (16%) had a combined type (lipoatrophy & lipohypertrophy), with 12 having severe features. Dyslipidemia (fasting hypercholesterolemia [HC] and/or hypertriglyceridemia [HT] defined according to age/sex adjusted thresholds) was present in 128 (29%, 95%CI 25.2-34.0) children, with 19 having HC only, 79 HT only and 30 both HC and HT. Median total cholesterol among HC cases was 226 mg/dl and median triglycerides among HT cases was 202 mg/dl. Seventy (16%, 95%CI 12.8-19.9) children had both dyslipidemia and body fat alterations, with a significant association between these factors overall (χ^2=5.29, p=0.021). Six (1.4%) children had impaired glucose tolerance.

Conclusions: LS is a relevant syndrome in HIV-infected children. Here, body fat changes affected nearly half and dyslipidemia around one-third of the patients, with 1 in 6 having both abnormalities, which were significantly associated. This ongoing study will allow description of risk factors in a large study population and evaluation of LS progression and management.

Appendix B Appendix to Chapter 2

B.1 Centres for Disease Control (CDC) clinical categories for children with human immunodeficiency virus (HIV) infection (reproduced from[581,582])

Category N: Not symptomatic

No signs or symptoms considered to the result of HIV infection or not to have any of the conditions listed in category A.

Category A: Mildly symptomatic

Two or more of the following conditions, but none of the conditions listed in categories B and C.

- Lymphadenopathy (≥0.5 cm at more than two sites; bilateral = one site)
- Hepatomegaly
- Splenomegaly
- Dermatitis
- Parotitis
- Recurrent or persistent upper respiratory infection, sinusitis, or otitis media

Category B: moderately symptomatic

Symptomatic conditions other than those listed for categories A or C that are attributable to HIV infection, e.g:

- Anaemia (<8 mg/dL), neutropenia (< 1000mm^3), or thrombocytopenia (<100000mm^3) persisting ≥30 days
- Bacterial meningitis, pneumonia, or sepsis (single episode)
- Candidiasis, oropharyngeal (thrush), persisting (>2 months) in children >6 months of age
- Cardiomyopathy
- Cytomegalovirus infection, with onset before 1 month of age
- Diarrheal, recurrent or chronic
- Hepatitis
- Herpes simplex virus (HSV) stomatitis, recurrent (more than two episodes within 1 year)
- HSV bronchitis, pneumonitis, or esophagitis with onset before 1 month of age

- Herpes zoster (shingles) involving at least two distinct episodes or more than one dermatome
- Leiomyosarcoma
- Lymphoid interstitial pneumonia (LIP) or pulmonary lymphoid hyperplasia complex
- Nephropathy
- Norcardiosis
- Persistent fever (lasting >1 month)
- Toxoplasmosis, onset before 1 month of age
- Varicella, disseminated (complicated chickenpox)

Category C: severely symptomatic

Any of the following:

- Serious bacterial infections, multiple or recurrent (i.e., any combination of at least two culture-confirmed infections within a 2-year period), of the following types: septicaemia, pneumonia, meningitis, bone or joint infection, or abscess of an internal organ or body cavity (excluding otitis media, superficial skin or mucosal abscesses, and indwelling catheter-related infections)
- Candidiasis, oesophageal or pulmonary (bronchi, trachea, lungs)
- Coccidiodomycosis, disseminated (at site other than or in addition to lungs or cervical or hilar lymph nodes)
- Cryptoococcosis, extrapulmonary
- Cryptosporidiosis or isosporiasis with diarrhoea persisting >1 month
- Cytomegalovirus disease with onset of the symptoms at age >1 month (at a site other than liver, spleen, or lymph nodes)
- Encephalopathy (at least one of the following progressive findings present for at least 2 months in the absence of a concurrent illness other than HIV infection that could explain the findings): (a) failure to attain or loss of developmental milestones or loss of intellectual ability, verified by standard developmental scale or neuropsychological tests; (b) impaired brain growth or acquired microcephaly demonstrated by head circumference measurements or brain atrophy demonstrated by computerized tomography or magnetic resonance imaging (serial imaging is required for children <2 years of age; (c) acquired symmetric motor deficit manifested by two or more of the following: paresis, pathologic reflexes, ataxia, or gait disturbance

- Herpes simplex virus infection causing a mucocutaneous ulcer that persists for >1 month: or bronchitis, pneumonitis, or esophagitis for any duration affecting a child >1 month of age
- Histoplasmosis, disseminated (at a site other than or in addition to lungs or cervical or hilar lymph nodes)
- Kaposi's sarcoma
- Lymphoma, primary, in brain
- Lymphoma, small, non-cleaved cell (Burkett's), or immunoblastic or large cell lymphoma of a B-cell or unknown immunologic phenotype
- *Mycobacterium tuberculosis*, disseminated or extra pulmonary
- *Mycobacterium*, other species or unidentified species, disseminated (at a site other than or in addition to lungs, skin or cervical or hilar lymph nodes)
- *Pneumocystis carinii* pneumonia
- Progressive multifocal leukoencephalopathy
- Salmonella (no typhoid) septicaemia, recurrent
- Toxoplasmosis of the brain with onset at >1 month of age
- Wasting syndrome in the absence of a concurrent illness other than HIV infection that could explain the following findings: (a) persistent weight loss >10% of baseline OR (b) downward crossing of at least two of the following percentile lines on the weight-for-age chart (e.g., 95^{th}, 7^{th}, 50^{th}, 25^{th}, 5^{th}) in a child ≥1 year of age OR (c) $<5^{th}$ percentile on weight-for-height chart on two consecutive measurements, ≥30 days apart PLUS (a) chronic diarrhoea (i.e., at least two loose stools per day for ≥30 days) OR (b) documented fever (for >30 days intermittent or constant).

B.2 Thresholds for hypercholesterolemia and fasting hypertriglyceridemia

Table B-1: Age and gender defined thresholds for hypercholesterolemia

Age (years)	Male (mg/dL)		Female (mg/dL)	
	Any	Moderate/ Severe	Any	Moderate/ Severe
2-11	≥200	≥240	≥200	≥240
12	≥233	≥280	≥212	≥254
13	≥225	≥270	≥209	≥251
14	≥220	≥264	≥208	≥250
15	≥220	≥264	≥211	≥253
16	≥223	≥268	≥217	≥260
17	≥227	≥272	≥225	≥270
18	≥233	≥280	≥233	≥280
19	≥238	≥286	≥239	≥287
20	≥240	≥288	≥240	≥288

Table B-2: Age and gender defined thresholds for fasting hypertriglyceridemia

Age (years)	Male (mg/dL)		Female (mg/dL)	
	Any	Moderate/ Severe	Any	Moderate/ Severe
2-9	≥100	≥120	≥100	≥120
10-11	≥130	≥156	≥130	≥156
12	≥163	≥196	≥180	≥216
13	≥171	≥205	≥171	≥205
14	≥179	≥215	≥161	≥193
15	≥186	≥223	≥158	≥190
16	≥191	≥229	≥162	≥194
17	≥195	≥234	≥172	≥206
18	≥198	≥238	≥185	≥222
19	≥200	≥240	≥196	≥235
20	≥200	≥240	≥200	≥240

B.3 Thresholds for immuno-suppression

Table B-3: Centres for disease control (CDC) defined categories of immunosuppression by age-specific CD4 T-lymphocyte counts and percentage of total lymphocytes (reproduced from[581,582])

	Age of child					
	<12 months		1-5 years		6-12 years	
	µL	%	µL	%	µL	%
No evidence of immuno-suppression	≥1 500	≥25	≥1 000	≥25	≥500	≥25
Moderate immuno-suppression	750 - 1 499	15-24	500 - 999	15-24	200 - 499	15-24
Severe immuno-suppression	<750	<15	<500	<15	<200	<15

B.4 Data collection form A

Body fat alterations and metabolic abnormalities in HIV-infected children and adolescents in Europe: a longitudinal study

Screening questionnaire for HIV-infected children not enrolled in the ECS

Child Identification number: ..... (Italian Register ID or hospital number)

Date form completed (dd/mm/yy): .....

Hospital:..... Paediatrician:.....

1. Baseline information

Child's date of birth (dd/mm/yy): ..... Gender: Male / Female

Ethnic group: White / Black / Other Country of birth: .....

Birth weight (g): ..... Birth length (cm) .....

Gestational age (weeks): .....

Child's most likely mode of HIV acquisition

Mother-to-child transmission Y / N Blood products / transfusion Y / N

Unknown Y / N Other Y / N

Was this child exposed to any antiretroviral drugs in utero?

No / Yes – monotherapy / Yes – dual therapy / Yes - HAART

If yes, when did the child's mother start taking antiretroviral drugs?

Before pregnancy / 1st trimester / 2nd trimester / 3rd trimester

Clinical, immunological and virological status

Which of the following describes the child's current HIV clinical status?

no symptoms / mild symptoms / moderate symptoms / severe symptoms

Maximum CDC clinical stage: N / A / B / C Date of onset (dd/mm/yy): .....

Most recent CD4 count (cells/mm^3): ..... Date (dd/mm/yy) .....

Most recent CD4 percentage (%): ..... Date (dd/mm/yy) .....

Nadir CD4 count (cells/mm^3) ..... Date (dd/mm/yy) .....

Most recent HIV-RNA (copies/ml): ..... Date (dd/mm/yy) .....

Is this child coinfected with HCV? Y / N

Antiretroviral treatment

Has the child **ever** received **any** antiretroviral therapy? No / Yes *(if yes, please specify below)*

Drug name	Date started	Date stopped	Currently taken?
			Yes / No
			Yes / No
			Yes / No
			Yes / No
			Yes / No
			Yes / No
			Yes / No
			Yes / No
			Yes / No
			Yes / No
			Yes / No
			Yes / No

Anthropometric measurements

For information on measuring waist and hip circumferences, please see the appendix in the protocol

Date of measurements (dd/mm/yy) ……

Weight (kg)		Height (cm)	
Waist circumference (cm)		Hip circumference (cm)	

Tanner score for pubertal stage (I - V): ……

Blood pressure (mm/Hg): **1.** …… **2.** …… **3.** ……
Please perform 3 measurements, ≥10 minutes apart (see protocol appendix)

Has the child ever had a bone fracture? No / Yes

If yes, please state which bone(s) and age (in years and months) at which the fracture(s) occurred:

……

Has the child ever experienced the following? If yes, please provide details:

Back pain ?

No / Yes If yes: Was this mild / moderate / severe?

Age at presentation (yrs & mths) _____

Movement impairment?

No / Yes If yes: Was this mild / moderate / severe?

Age at presentation (yrs & mths) _____

Lipid and glucidic profile

Please provide results, if available:

Date blood drawn (dd/mm/yy) .....

	Please ring:
Total cholesterol (mg/dl):	Fasting / non-fasting
HDL cholesterol (mg/dl):	Fasting / non-fasting
LDL cholesterol (mg/dl):	Fasting / non-fasting
Triglycerides (mg/dl):	Fasting / non-fasting
Glucose (mg/dl):	Fasting / non-fasting
Insulin (mcU/ml):	Fasting / non-fasting

Other tests: If this child has had any other tests such as **oral glucose tolerance test, magnetic resonance imaging or DEXA** please could you attach copies of the results to this form.

Family history

We would like to know whether there is evidence of a genetic predisposition for dyslipidemia in the child's family. Does the child's biological mother, father or grandparents have a history of any of the following conditions: early onset Type I or Type II diabetes, hypertension, cardio-vascular events, high cholesterol/triglycerides?

If yes, please specify the condition / event, the age of onset and family member: .....

.....

.....

Does the child's biological mother or father have HIV-associated lipodystrophy syndrome?

	Not known	Not applicable	No	Fat gain	Fat loss	Dyslipidemia
Mother						
Father						

2. Lipodystrophy syndrome screening questions

a. Body fat redistribution

Please complete the following table, with one tick (√) per row. "Mild" symptoms are defined as those only noticeable when specifically inspected, "moderate" as readily obvious to the child / carer and "severe" as obvious to a casual observer.

	Normal	Mild	Moderate	Severe
Fat loss Face (sunken cheeks, sunken eyes, prominent zygomatic arch)				
Arms (skinny; prominent veins, muscularity and bones)				
Legs (skinny; prominent veins, muscularity and bones)				
Buttocks (loose skin folds, prominent muscles, loss of contour and fat, hollowing)				
Fat accumulation Trunk (increase in abdominal girth)				
Base of neck / back ("buffalo hump")				
Breast enlargement				

b. Dyslipidemia and glucose disorders

Please use the following tables to identify whether this child has dyslipidemia:

If the child has a cholesterol level at or above the thresholds appropriate for age and sex, as provided in the following table then this child is considered to have hypercholesterolemia.

Age	Male		Female	
	mmol/L	mg/dl	mmol/L	mg/dl
2-11 years	≥5.18	≥200	≥5.18	≥200
12 years	≥6.03	≥233	≥5.47	≥212
13 years	≥5.83	≥225	≥5.41	≥209
14 years	≥5.70	≥220	≥5.38	≥208
15 years	≥5.70	≥220	≥5.46	≥211
16 years	≥5.77	≥223	≥5.62	≥217
17 years	≥5.88	≥227	≥5.82	≥225
18 years	≥6.02	≥233	≥6.03	≥233
19 years	≥6.16	≥238	≥6.17	≥239
20 years	≥6.22	≥240	≥6.22	≥240

If the child has a triglyceride level at or above the threshold appropriate for age and sex, as provided in the following table then this child is considered to have hypertriglyceridemia.

Age	Male		Female	
	mmol/L	mg/dl	mmol/L	mg/dl
2-9 years	≥1.13	≥100	≥1.13	≥100
10-11 years	≥1.47	≥130	≥1.47	≥130
12 years	≥1.84	≥163	≥2.03	≥180
13 years	≥1.93	≥171	≥1.93	≥171
14 years	≥2.02	≥179	≥1.82	≥161
15 years	≥2.10	≥186	≥1.79	≥158
16 years	≥2.16	≥191	≥1.83	≥162
17 years	≥2.20	≥195	≥1.94	≥172
18 years	≥2.24	≥198	≥2.09	≥185
19 years	≥2.26	≥200	≥2.22	≥196
20 years	≥2.26	≥200	≥2.26	≥200

Does the child have:		
Hypercholesterolemia (according to the table above)	No ☐	Yes ☐
Hypertriglyceridemia (according to the table above)	No ☐	Yes ☐
Impaired glucose tolerance i.e. impaired fasting glucose: 110-125 mg/dl or impaired glucose tolerance (fasting glucose <126 mg/dl and glucose value 2 hours post oral glucose tolerance test of 140-199 mg/dl)	No ☐	Yes ☐

If this child has **one or more** of the above symptoms of fat redistribution or dyslipidemia / glucose disorders (that is, if any of the shaded boxes have been ticked), we consider this child to have **lipodystrophy syndrome.**

If this child does not currently have any symptoms from the above lists (that is, if only the non-shaded boxes have been ticked), we consider this child **NOT** to have lipodystrophy syndrome at this time.

What happens next?

- **If this child does NOT have lipodystrophy at this time**
  - You have completed all the necessary questions
  - Please return this form to the coordinating centre. We will contact you again about this child in 6 months time to re-assess their lipodystrophy status.
- **If this child has lipodystrophy**
  - Please complete the final section below and then return the form to the appropriate coordinating centre.

3. Children with lipodystrophy

Management of lipodystrophy syndrome

Has the child ever been treated with any of the following: rhGH, lipid lowering agents (e.g. statin, fibrate, resin), sexual hormones, steroids, insulin-sensitizing agents (e.g. metformin)?

No / Yes* **If yes, please complete the table below*

DRUG NAME AND DAILY DOSE	START (dd/mm/yy)	STOP (dd/mm/yy)	Currently taken?
			No / Yes
			No / Yes
			No / Yes
			No / Yes
			No / Yes

Have any of the following approaches been used to manage the child's lipodystrophy symptoms?
**If yes, please give details:*

- Specific dietary interventions No / Yes* .....
- Increased physical activity No / Yes* .....
- Surgery No / Yes* .....

Thank you for completing this questionnaire. Please return to Prof Alessandra Vigano, Clinica Pediatrica, Ospedale L.Sacco, Via Grassi 74, 20157 Milano, Italy or Dr Claire Thorne, MRC Centre of Epidemiology for Child Health, Institute of Child Health, 30 Guilford Street, London, WC1N 1EH, UK. Fax: +44 20 7813 8145

B.5 Original data collection form B

Form B / Oth

Body fat alterations and metabolic abnormalities in HIV-infected children and adolescents in Europe: a longitudinal study

Follow-up questionnaire for Lipodystrophy cases (non-ECS children)

Child Identification number: ..... (Italian Register ID or hospital number)

Date form completed (dd/mm/yy): .....

Hospital:..... Paediatrician:.....

1. Clinical, immunological and virological status

Current CDC clinical stage: N / A / B / C

Date blood drawn: .....

Most recent CD4 count (cells/mm^3): ..... Most recent CD4 %: .....

Most recent HIV-RNA (copies/ml): .....

2. Body fat redistribution

Please complete the following table, with one tick (√) per row. "Mild" symptoms are defined as those only noticeable when specifically inspected, "moderate" as readily obvious to the child / carer and "severe" as obvious to a casual observer.

	Normal	Mild	Moderate	Severe
Fat loss Face (sunken cheeks, sunken eyes, prominent zygomatic arch)				
Arms (skinny; prominent veins, muscularity and bones)				
Legs (skinny; prominent veins, muscularity and bones)				
Buttocks (loose skin folds, prominent muscles, loss of contour and fat, hollowing)				
Fat accumulation Trunk (increase in abdominal girth)				
Base of neck / back ("buffalo hump")				
Breast enlargement				

3. Anthropometric measurements

Date of measurements (dd/mm/yy) .....

Weight (kg)		Height (cm)	
Waist circumference (cm)		Hip circumference (cm)	

Tanner score for pubertal stage (I - V): .....

1

Blood pressure (mm/Hg): **1.** ..... **2.** ..... **3.** .....
Please perform 3 measurements, ≥10 minutes apart (see protocol appendix)

4. Lipid and glucidic profile

Please provide results, if available for the following:

Date blood drawn (dd/mm/yy) .....

	Please ring:
Total cholesterol (mg/dl):	Fasting / non-fasting
HDL cholesterol (mg/dl):	Fasting / non-fasting
LDL cholesterol (mg/dl):	Fasting / non-fasting
Triglycerides (mg/dl):	Fasting / non-fasting
Glucose (mg/dl):	Fasting / non-fasting
Insulin (mcU/ml):	Fasting / non-fasting

5. Other tests: If this child has had any other tests such as **oral glucose tolerance test, magnetic resonance imaging or DEXA** please could you attach copies of the results to this form.

6. Antiretroviral therapy

How has this child's antiretroviral therapy changed over the past 6 months?

- No change (same regimen) ☐
- All ARV treatment stopped ☐ → Stop date (dd/mm/yy) .....
- Regimen modified ☐ → *Please complete the table below*

Please give standardised drug name (one drug per row) with Start date, and Stop date if applicable. If drug is currently taken, leave Stop date blank. In "Reasons for stopping" box, please use appropriate letter code(s) (see coding table below) and specify all applicable reasons.

ARV DRUG AND DAILY DOSE	START DATE	STOP DATE	Reason(s) for stopping

Reason for stopping			
Body composition changes	**A**	Treatment failure	**C**

Dyslipidemia	**B**	Other	**D**

7. Management of lipodystrophy syndrome

Has the child ever been treated with any of the following: rhGH, lipid lowering agents (e.g. statin, fibrate, resin), sexual hormones, steroids, insulin-sensitizing agents (e.g. metformin)?

No / Yes* **If yes, please complete the table below*

DRUG NAME AND DAILY DOSE	START (dd/mm/yy)	STOP (dd/mm/yy)	Currently taken?
			No / Yes
			No / Yes
			No / Yes
			No / Yes
			No / Yes
			No / Yes
			No / Yes
			No / Yes

Have any of the following approaches been used to manage the child's lipodystrophy symptoms?
**If yes, please give details:*

- Specific dietary interventions No / Yes* .....
- Increased physical activity No / Yes* .....
- Surgery No / Yes* .....

Thank you for completing this questionnaire.

Please return to Prof Alessandra Vigano, Clinica Pediatrica, Ospedale L.Sacco, Via Grassi 74, 20157 Milano, Italy.

B.6 Revised data collection form B

Body fat alterations and metabolic abnormalities in HIV-infected children and adolescents in Europe: a longitudinal study

Follow-up questionnaire for Lipodystrophy cases (non-ECS children)

Child Identification number: ..... (Italian Register ID or hospital number)

Date form completed (dd/mm/yy): .....

Hospital:..... Paediatrician:.....

1. Clinical, immunological and virological status

Current CDC clinical stage: N / A / B / C

Date blood drawn: .....

Most recent CD4 count (cells/mm^3): ..... Most recent CD4 %: .....

Most recent HIV-RNA (copies/ml): .....

2. Body fat redistribution

Please complete the following table, with one tick (√) per row. "Mild" symptoms are defined as those only noticeable when specifically inspected, "moderate" as readily obvious to the child / carer and "severe" as obvious to a casual observer.

	Normal	Mild	Moderate	Severe
Fat loss Face (sunken cheeks, sunken eyes, prominent zygomatic arch)				
Arms (skinny; prominent veins, muscularity and bones)				
Legs (skinny; prominent veins, muscularity and bones)				
Buttocks (loose skin folds, prominent muscles, loss of contour and fat, hollowing)				
Fat accumulation Trunk (increase in abdominal girth)				
Base of neck / back ("buffalo hump")				
Breast enlargement				

3. Anthropometric measurements

Date of measurements (dd/mm/yy) .....

Weight (kg)		Height (cm)	
Waist circumference (cm)		Hip circumference (cm)	

Tanner score for pubertal stage (I - V): .....

Age at menarche..... Not applicable: *No/Yes*

Blood pressure (mm/Hg): **1.**..... **2.** ..... **3.** .....
Please perform 3 measurements, ≥10 minutes apart (see protocol appendix)

4. Lab tests

Please provide results, if available for the following:

Date blood drawn (dd/mm/yy) .....

	Please ring:
Total cholesterol (mg/dL):	Fasting / non-fasting
HDL cholesterol (mg/dL):	Fasting / non-fasting
LDL cholesterol (mg/dL):	Fasting / non-fasting
Triglycerides (mg/dL):	Fasting / non-fasting
Glucose (mg/dL):	Fasting / non-fasting
Insulin (mcU/mL):	Fasting / non-fasting
Creatine (mg/dl):	Fasting / non-fasting

AST; aspartate transaminase (IU/L).....

ALT; alanine transaminase (IU/L) .....

5. Other tests: If this child has had any other tests such as **oral glucose tolerance test, magnetic resonance imaging or DEXA** please could you attach copies of the results to this form.

6. Antiretroviral therapy

How has this child's antiretroviral therapy changed over the past 6 months?

- No change (same regimen) ☐
- All ARV treatment stopped ☐ → Stop date (dd/mm/yy) .....
- Regimen modified ☐ → *Please complete the table below*

Please give standardised drug name (one drug per row) with Start date, and Stop date if applicable. If drug is currently taken, leave Stop date blank. In "Reasons for stopping" box, please use appropriate letter code(s) (see coding table below) and specify all applicable reasons.

ARV DRUG AND DAILY DOSE	START DATE	STOP DATE	Reason(s) for stopping

Reason for stopping			
Body composition changes	A	Treatment failure	C
Dyslipidemia	B	Other	D

7. Concurrent medications

Please give details of any concurrent medications or vitamin/mineral supplements

8. Behavior

Smoking	Ever tried a cigarette	No/Yes
	Regular smoker	No/Yes
	How many cigarettes smoked in the last week	.....
Alcohol	Ever had a (whole) alcoholic drink	No/Yes
	Regularly drinks	No/Yes
	How many days where alcohol was drunk in the last week	.....

9. Management of lipodystrophy syndrome

Has the child ever been treated with any of the following: rhGH, lipid lowering agents (e.g. statin, fibrate, resin), sexual hormones, steroids, insulin-sensitizing agents (e.g. metformin)?

No / Yes* **If yes, please complete the table below*

DRUG NAME AND DAILY DOSE	START (dd/mm/yy)	STOP (dd/mm/yy)	Currently taken?
			No / Yes
			No / Yes
			No / Yes
			No / Yes
			No / Yes
			No / Yes
			No / Yes
			No / Yes

Have any of the following approaches been used to manage the child's lipodystrophy symptoms? **If yes, please give details:*

- Specific dietary interventions No / Yes* .....
- Increased physical activity No / Yes* .....
- Surgery No / Yes* .....

Thank you for completing this questionnaire

B.7 Original data collection form C

Form C / Oth

Body fat alterations and metabolic abnormalities in HIV-infected children and adolescents in Europe: a longitudinal study

Screening questionnaire for HIV-infected children not enrolled in the ECS

Child Identification number: …… (Italian Register ID or hospital number)

Child's date of birth …… Date form completed (dd/mm/yy): ……

Hospital:…… Paediatrician:……

Clinical, immunological and virological status

Which of the following describes the child's current HIV clinical status?

no symptoms / mild symptoms / moderate symptoms / severe symptoms

Most recent CD4 count (cells/mm^3): …… Date (dd/mm/yy) ……

Most recent CD4 percentage (%): …… Date (dd/mm/yy) ……

Most recent HIV-RNA (copies/ml): …… Date (dd/mm/yy) ……

Anthropometric measurements

Date of measurements (dd/mm/yy) ……

Weight (kg)		Height (cm)	
Waist circumference (cm)		Hip circumference (cm)	

Tanner score for pubertal stage (I - V): ……

Blood pressure (mm/Hg): **1.** …… **2.** …… **3.** ……
Please perform 3 measurements, ≥10 minutes apart (see protocol appendix)

Lipid and glucidic profile: ***Please provide results, if available:***

Date blood drawn (dd/mm/yy) ……

Please ring:

Total cholesterol (mg/dl): …… Fasting / non-fasting

HDL cholesterol (mg/dl): …… Fasting / non-fasting

LDL cholesterol (mg/dl): …… Fasting / non-fasting

Triglycerides (mg/dl): …… Fasting / non-fasting

Glucose (mg/dl): …… Fasting / non-fasting

Insulin (mcU/ml): …… Fasting / non-fasting

Other tests: If this child has had any other tests such as **oral glucose tolerance test, magnetic resonance imaging or DEXA** please could you attach copies of the results to this form.

1

Antiretroviral therapy

How has this child's antiretroviral therapy changed over the past 6 months?

- No change (same regimen) ☐
- All ARV treatment stopped ☐ → Stop date (dd/mm/yy) .....
- Regimen modified ☐ → *Please complete the table below*

Please give standardised drug name (one drug per row) with Start date, and Stop date if applicable. If drug is currently taken, leave Stop date blank. In "Reasons for stopping" box, please use appropriate letter code(s) (see coding table below) and specify all applicable reasons.

ARV DRUG AND DAILY DOSE	START DATE	STOP DATE	Reason(s) for stopping

*Reason for stopping: **1.** body composition changes, **2.** dyslipidemia; **3.** treatment failure (ie virological, immunological and/or clinical failure); **4.** other toxicity; **5.** patient's/parent's wish; **6.** structured treatment interruption; **7.** other

Lipodystrophy syndrome screening questions

a. Body fat redistribution

Please complete the following table, with one tick (√) per row. "Mild" symptoms are defined as those only noticeable when specifically inspected, "moderate" as readily obvious to the child / carer and "severe" as obvious to a casual observer.

	Normal	Mild	Moderate	Severe
Fat loss Face (sunken cheeks, sunken eyes, prominent zygomatic arch)				
Arms (skinny; prominent veins, muscularity and bones)				
Legs (skinny; prominent veins, muscularity and bones)				
Buttocks (loose skin folds, prominent muscles, loss of contour and fat, hollowing)				
Fat accumulation Trunk (increase in abdominal girth)				
Base of neck / back ("buffalo hump")				
Breast enlargement				

b. Dyslipidemia and glucose disorders
Please use the following tables to identify whether this child has dyslipidemia:

If the child has a cholesterol level at or above the thresholds appropriate for age and sex, as provided in the following table then this child is considered to have hypercholesterolemia.

Age	Male		Female	
	mmol/L	mg/dl	mmol/L	mg/dl
2-11 years	≥5.18	≥200	≥5.18	≥200
12 years	≥6.03	≥233	≥5.47	≥212
13 years	≥5.83	≥225	≥5.41	≥209
14 years	≥5.70	≥220	≥5.38	≥208
15 years	≥5.70	≥220	≥5.46	≥211
16 years	≥5.77	≥223	≥5.62	≥217
17 years	≥5.88	≥227	≥5.82	≥225
18 years	≥6.02	≥233	≥6.03	≥233
19 years	≥6.16	≥238	≥6.17	≥239
20 years	≥6.22	≥240	≥6.22	≥240

If the child has a triglyceride level at or above the threshold appropriate for age and sex, as provided in the following table then this child is considered to have hypertriglyceridemia.

Age	Male		Female	
	mmol/L	mg/dl	mmol/L	mg/dl
2-9 years	≥1.13	≥100	≥1.13	≥100
10-11 years	≥1.47	≥130	≥1.47	≥130
12 years	≥1.84	≥163	≥2.03	≥180
13 years	≥1.93	≥171	≥1.93	≥171
14 years	≥2.02	≥179	≥1.82	≥161
15 years	≥2.10	≥186	≥1.79	≥158
16 years	≥2.16	≥191	≥1.83	≥162
17 years	≥2.20	≥195	≥1.94	≥172
18 years	≥2.24	≥198	≥2.09	≥185
19 years	≥2.26	≥200	≥2.22	≥196
20 years	≥2.26	≥200	≥2.26	≥200

Does the child have:
Hypercholesterolemia (according to the table above) No ☐ Yes ☐

Hypertriglyceridemia (according to the table above) No ☐ Yes ☐

Impaired glucose tolerance No ☐ Yes ☐
i.e. impaired fasting glucose: 110-125 mg/dl or impaired glucose tolerance (fasting glucose <126 mg/dl and glucose value 2 hours post oral glucose tolerance test of 140-199 mg/dl)

If this child has **one or more** of the above symptoms of fat redistribution or dyslipidemia / glucose disorders (that is, if any of the shaded boxes have been ticked), we consider this child to have **lipodystrophy syndrome.**

If this child does not currently have any symptoms from the above lists (that is, if only the non-shaded boxes have been ticked), we consider this child **NOT** to have lipodystrophy syndrome at this time.

What happens next?

- **If this child does NOT have lipodystrophy at this time**
  - You have completed all the necessary questions
  - Please return this form to the coordinating centre. We will contact you again about this child in 6 months time to re-assess their lipodystrophy status.
- **If this child has lipodystrophy**
  - Please complete the final section below and then return the form to the appropriate coordinating centre.

3. Children with lipodystrophy

Management of lipodystrophy syndrome

Has the child ever been treated with any of the following: rhGH, lipid lowering agents (e.g. statin, fibrate, resin), sexual hormones, steroids, insulin-sensitizing agents (e.g. metformin)?

No / Yes* **If yes, please complete the table below*

DRUG NAME AND DAILY DOSE	START (dd/mm/yy)	STOP (dd/mm/yy)	Currently taken?
			No / Yes
			No / Yes
			No / Yes
			No / Yes
			No / Yes
			No / Yes
			No / Yes
			No / Yes

Have any of the following approaches been used to manage the child's lipodystrophy symptoms?
**If yes, please give details:*

- Specific dietary interventions No / Yes* .....
- Increased physical activity No / Yes* .....
- Surgery No / Yes* .....

Thank you for completing this questionnaire.

Please return to:
Prof Alessandra Vigano, Clinica Pediatrica, Ospedale L.Sacco, Via Grassi 74, 20157 Milano, Italy
or
Dr Claire Thorne, MRC Centre of Epidemiology for Child Health, Institute of Child Health, 30 Guilford Street, London, WC1N 1EH, UK. c.thorne@ich.ucl.ac.uk Fax: +44 20 7813 8145

B.8 Revised data collection form C

Body fat alterations and metabolic abnormalities in HIV-infected children and adolescents in Europe: a longitudinal study

Screening questionnaire for HIV-infected children not enrolled in the ECS

Child Identification number: ..... (Italian Register ID or hospital number)

Child's date of birth .....

Date form completed (dd/mm/yy): .....

Hospital:..... Paediatrician:.....

I. Background data questions

1. Clinical, immunological and virological status

Current CDC clinical stage: N / A / B / C

Most recent CD4 count (cells/mm^3): ..... Date (dd/mm/yy) .....

Most recent CD4 percentage (%): ..... Date (dd/mm/yy) .....

Most recent HIV-RNA (copies/ml): ..... Date (dd/mm/yy) .....

2. Anthropometric measurements

Date of measurements (dd/mm/yy) .....

Weight (kg)		Height (cm)	
Waist circumference (cm)		Hip circumference (cm)	

Tanner score for pubertal stage (I - V): .....

Age at menarche..... Not applicable: *No/Yes*

Blood pressure (mm/Hg): **1.**..... **2.** ..... **3.** .....
Please perform 3 measurements, ≥10 minutes apart (see protocol appendix)

3. Lab tests

Please provide results, if available:

Date blood drawn (dd/mm/yy) .....

	Please ring:
Total cholesterol (mg/dl):	Fasting / non-fasting
HDL cholesterol (mg/dl):	Fasting / non-fasting
LDL cholesterol (mg/dl):	Fasting / non-fasting
Triglycerides (mg/dl):	Fasting / non-fasting
Glucose (mg/dl):	Fasting / non-fasting
Insulin (mcU/ml):.....	Fasting / non-fasting
Creatine (mg/dl):	Fasting / non-fasting

AST; aspartate transaminase (IU/L).....

ALT; alanine transaminase (IU/L) .....

4. Antiretroviral therapy

How has this child's antiretroviral therapy changed over the past 6 months?

- No change (same regimen) ☐
- All ARV treatment stopped ☐ → Stop date (dd/mm/yy) .....
- Regimen modified ☐ → *Please complete the table below*

Please give standardised drug name (one drug per row) with Start date, and Stop date if applicable. If drug is currently taken, leave Stop date blank. In "Reasons for stopping" box, please use appropriate letter code(s) (see coding table below) and specify all applicable reasons.

ARV DRUG AND DAILY DOSE	START DATE	STOP DATE	Reason(s) for stopping

Reason for stopping	
Body composition changes	A
Dyslipidemia	B
Treatment failure	C
Other	D

5. Concurrent medications

Please give details of any concurrent medications or vitamin/mineral supplements

6. Behavior

Smoking	Ever tried a cigarette	No/Yes
	Regular smoker	No/Yes
	How many cigarettes smoked in the last week	.....
Alcohol	Ever had a (whole) alcoholic drink	No/Yes
	Regularly drinks	No/Yes
	How many days where alcohol was drunk in the last week	.....

II. Lipodystrophy syndrome screening questions

7. Body fat redistribution

Please complete the following table, with one tick (√) per row. "Mild" symptoms are defined as those only noticeable when specifically inspected, "moderate" as readily obvious to the child / carer and "severe" as obvious to a casual observer.

	Normal	Mild	Moderate	Severe
Fat loss Face (sunken cheeks, sunken eyes, prominent zygomatic arch)				
Arms (skinny; prominent veins, muscularity and bones)				
Legs (skinny; prominent veins, muscularity and bones)				
Buttocks (loose skin folds, prominent muscles, loss of contour and fat, hollowing)				
Fat accumulation Trunk (increase in abdominal girth)				
Base of neck / back ("buffalo hump")				
Breast enlargement				

8. Dyslipidemia and glucose disorders

Please use the following tables to identify whether this child has dyslipidemia:

If the child has a cholesterol level at or above the thresholds appropriate for age and sex, as provided in the following table then this child is considered to have hypercholesterolemia.

Age	Male		Female	
	mmol/L	mg/dl	mmol/L	mg/dl
2-11 years	≥5.18	≥200	≥5.18	≥200
12 years	≥6.03	≥233	≥5.47	≥212
13 years	≥5.83	≥225	≥5.41	≥209
14 years	≥5.70	≥220	≥5.38	≥208
15 years	≥5.70	≥220	≥5.46	≥211
16 years	≥5.77	≥223	≥5.62	≥217
17 years	≥5.88	≥227	≥5.82	≥225
18 years	≥6.02	≥233	≥6.03	≥233
19 years	≥6.16	≥238	≥6.17	≥239
20 years	≥6.22	≥240	≥6.22	≥240

If the child has a triglyceride level at or above the threshold appropriate for age and sex, as provided in the following table then this child is considered to have hypertriglyceridemia.

Age	Male		Female	
	mmol/L	mg/dl	mmol/L	mg/dl
2-9 years	≥1.13	≥100	≥1.13	≥100
10-11 years	≥1.47	≥130	≥1.47	≥130
12 years	≥1.84	≥163	≥2.03	≥180
13 years	≥1.93	≥171	≥1.93	≥171
14 years	≥2.02	≥179	≥1.82	≥161
15 years	≥2.10	≥186	≥1.79	≥158
16 years	≥2.16	≥191	≥1.83	≥162
17 years	≥2.20	≥195	≥1.94	≥172
18 years	≥2.24	≥198	≥2.09	≥185
19 years	≥2.26	≥200	≥2.22	≥196
20 years	≥2.26	≥200	≥2.26	≥200

Does the child have:

Hypercholesterolemia (according to the table above) No ☐ Yes ☐

Hypertriglyceridemia (according to the table above) No ☐ Yes ☐

Impaired glucose tolerance No ☐ Yes ☐

i.e. impaired fasting glucose: 110-125 mg/dl or impaired glucose tolerance (fasting glucose <126 mg/dl and glucose value 2 hours post oral glucose tolerance test of 140-199 mg/dl)

Other tests: If this child has had any other tests such as **oral glucose tolerance test, magnetic resonance imaging or DEXA** please could you attach copies of the results to this form.

If this child has **one or more** of the above symptoms of fat redistribution or dyslipidemia / glucose disorders (that is, if any of the shaded boxes have been ticked), we consider this child to have **lipodystrophy syndrome.**

If this child does not currently have any symptoms from the above lists (that is, if only the non-shaded boxes have been ticked), we consider this child **NOT** to have lipodystrophy syndrome at this time.

What happens next?

- **If this child does NOT have lipodystrophy at this time**
  - You have completed all the necessary questions
  - Please return this form to the coordinating centre. We will contact you again about this child in 6 months time to re-assess their lipodystrophy status.
- **If this child has lipodystrophy**
  - Please complete the final section below and then return the form to the coordinating centre.

III. Children with lipodystrophy

9. Management of lipodystrophy syndrome

Has the child ever been treated with any of the following: rhGH, lipid lowering agents (e.g. statin, fibrate, resin), sexual hormones, steroids, insulin-sensitizing agents (e.g. metformin)?

No / Yes* **If yes, please complete the table below*

DRUG NAME AND DAILY DOSE	START (dd/mm/yy)	STOP (dd/mm/yy)	Currently taken?
			No / Yes
			No / Yes
			No / Yes
			No / Yes
			No / Yes
			No / Yes
			No / Yes
			No / Yes

Have any of the following approaches been used to manage the child's lipodystrophy symptoms?
**If yes, please give details:*

- Specific dietary interventions No / Yes* .....
- Increased physical activity No / Yes* .....
- Surgery No / Yes* .....

Thank you for completing this questionnaire

Appendix C Appendix to chapter 3

Note on box plots

There may not be agreement in the total number of data points when the same data is being displayed in box-plots (for medians) and scatter plots (for locally weighted smoothing and fractional polynomial models). This is because STATA automatically excludes "outside values" in box plots, i.e. box the length of the whiskers is restricted to 1.5 x the interquartile range. However, these exclusions only occur in the visual representations: no values are excluded in tests investigating statistical differences in medians.

C.1 Missing data

Table C-1: Missing physical and metabolic data by lipodystrophy outcome

		Total cholesterol			LDL cholesterol			Non-HDL cholesterol			HDL cholesterol			Waist/hip ratio		
		%	n/N	p-value	%	n/N	p-value	%	n/N	p-value	%	n/N	p-value	%	n/N	p-value
Ethnicity	**Black**	1.0	1/107	0.624	13.1	14/107		13.1	14/107		13.1	14/107	0.696	18.7	20/107	0.044
	White	2.1	6/285		19.6	56/285		16.1	46/285		16.1	46/285		29.1	83/285	
	Other	0.0	0/17		11.8	2/17		11.8	2/17		11.8	2/17		11.8	2/17	
Country	**Belgium**	1.2	1/84	0.430	2.4	2/84	<0.001	15.9	44/276	<0.001	15.9	44/276	<0.001	39.1	108/276	<0.001
	Italy	2.2	6/276		19.2	53/276		3.6	3/84		3.6	3/84		0.0	0/84	
	Poland	0.0	0/66		33.3	22/66		28.8	19/66		28.8	19/66		1.5	1/66	
Hospital	**Bambin Gesu**	2.1	1/47	0.405	2.1	1/47	<0.001	2.1	1/47	<0.001	2.1	1/47	<0.001	97.9	46/47	<0.001
	Como	0.0	0/3		0.0	0/3		0.0	0/3		0.0	0/3		33.3	1/3	
	Frederico II Napoli	0.0	0/34		11.8	4/34		11.8	4/34		11.8	4/34		5.9	2/34	
	Genova	0.0	0/27		0.0	0/27		0.0	0/27		0.0	0/27		3.7	1/27	
	Meyer	0.0	0/16		18.8	3/16		18.8	3/16		18.8	3/16		0.0	0/16	
	Padova	6.9	2/29		79.3	23/29		75.9	22/29		75.9	22/29		96.6	28/29	
	S. Matteo Pavia	0.0	0/6		8.3	5/6		16.7	1/6		16.7	1/6		0.0	0/6	
	Sacco	5.7	3/53		7.5	4/53		5.7	3/53		5.7	3/53		9.4	5/53	
	Torino	0.0	0/25		12.0	3/25		0.0	0/25		0.0	0/25		0.0	0/25	
	Civili Brescia	0.0	0/25		40.0	10/25		40.0	10/25		40.0	10/25		96.0	24/25	
	S. Paolo	0.0	0/11		0.0	0/11		0.0	0/11		0.0	0/11		9.1	1/11	
	St. Pierre	2.2	1/45		4.4	2/45		4.4	2/45		4.4	2/45		0.0	0/45	
	Liege	0.0	0/25		0.0	0/25		4.0	1/25		4.0	1/25		0.0	0/25	
	St. Luc	0.0	0/14		0.0	0/14		0.0	0/14		0.0	0/14		0.0	0/14	
	Warsaw	0.0	0/66		33.3	22/66		28.8	19/66		28.8	19/66		1.5	1/66	

Associations tested with χ^2 test

C.2 Birth length and birth weight regression analysis

Table C-2: Regression of birth length on body weight in subjects with birth gestation ≥ 37 weeks

	No body fat alterations *n* = 65		Body fat alterations *n* = 59	
	Estimate	***p* - value**	**Estimate**	***p* - value**
Slope	0.10	<0.001	0.10	<0.001
Intercept	-1.78	0.011	-1.74	0.196
Mean square of residuals	0.29		0.48	

C.3 Use of specific antiretroviral drugs at recruitment

Table C-3: Distribution of ever-use and current-use (at recruitment) of specific antiretroviral drugs

ART class	Drug	Ever use	Never use	Current use	Not current use
NRTI	**Lamivudine**	376	49	247	146
	Zidovudine	308	117	102	291
	Stavudine	218	207	44	349
	Didanosine	197	228	47	346
	Abacavir	176	249	118	275
	Tenofovir	126	299	103	290
	Emtricitabine	48	377	42	351
	Zalcitabine	30	395	0	393
	Kivexa	6	419	5	388
	Trizivir	2	423	1	392
NNRTI	**Efavirenz**	159	266	94	299
	Nevirapine	105	320	39	354
PI	**Ritonavir/kaletra**	257	168	197	196
	Nelfinavir	204	221	1	392
	Fosamprenavir	33	393	20	373
	Atazanavir	27	398	17	376
	Indinavir	26	399	1	392
	Saquinavir	19	406	0	393
	Tipranavir	4	421	3	390
FI	**Enfuviritide**	3	422	1	392

C.4 Fractional polynomial models stratified by sex

Table C-4: Fractional polynomial models fitting anthropometric and metabolic outcomes as a function of age at recruitment stratified by sex

Outcome	Sex	Model	*n*	Coefficients			
				Parameter	Estimate	95% confidence interval	*p*-value
Body mass index	**Male**	$y_i = \alpha_i + \beta_{1i}\ln(x_i) + \beta_{2i}\sqrt[2]{x_i}$	195	α	18.41	(17.91, 18.91)	<0.001
				β_1	-7.20	(-15.65, 1.26)	0.095
				β_2	23.63	(5.90, 41.36)	0.009
	Female	$y_i = \alpha_i + {\beta_{1i}}/{x_i^2} + \beta_{2i}ln(x_i)$	213	α	19.55	(19.08, 20.01)	<0.001
				β_1	0.91	(5.80, 9.98)	<0.001
				β_2	19.55	(19.08, 20.02)	<0.001
Wait/hip ratio	**Male**	$y_i = \alpha_i + \beta_{1i}x_i + \beta_{2i}x_i$	146	α	0.95	(0.93, 0.98)	<0.001
				β_1	0.09	(-0.14, 0.10)	0.272
				β_2	-0.13	(-0.35, 0.10)	0.272
	Female	$y_i = \alpha_i + \beta_{1i}x_i + \beta_{2i}x_i^2$	164	α	0.90	(0.88, 0.91)	<0.001
				β_1	-0.09	(-0.25, 0.07)	0.250
				β_2	0.02	(-0.25, 0.07)	0.609

Outcome	Sex	Model	*n*	Parameter	Estimate	95% confidence interval	Coefficients *p*-value
Total cholesterol	**Male**	$y_i = \alpha_i + {\beta_{1i}}/{x_i^2} + \beta_{2i}x_i^2$	197	α	167.12	(159.83, 174.41)	<0.001
				β_1	4.96	(0.63, 9.30)	0.025
				β_2	-2.67	(-7.20, 1.85)	0.245
	Female	$y_i = \alpha_i + \beta_{1i}x_i + \beta_{2i}x_i ln(x_i)$	211	α	170.95	(164.00, 177.91)	<0.001
				β_1	26.90	(-43.41, 97.22)	0.284
				β_2	-35.57	(-100.88, 29.74)	0.284
Low density lipoprotein (LDL) cholesterol	**Male**	$y_i = \alpha_i + {\beta_{1i}}/{x_i^2} + \beta_{2i}x_i$	158	α	90.23	(84.99, 95.47)	<0.001
				β_1	4.79	(0.53, 9.04)	0.851
				β_2	-1.82	(84.99, 95.47)	<0.001
	Female	$y_i = \alpha_i + {\beta_{1i}}/{x_i^2} + \beta_{2i}x_i$	183	α	95.16	(90.7, 99.66)	<0.001
				β_1	-77.29	(-132.70, -21.89)	0.007
				β_2	190.99	(60.83, 321.45)	0.004
Non-high density lipoprotein (HDL)	**Male**	$y_i = \alpha_i + \frac{\beta_{1i}}{x_i^2} + \frac{\beta_{2i}}{x_i^2}(ln(x_i))$	163	α	113.76	(107.87, 119.64)	<0.001

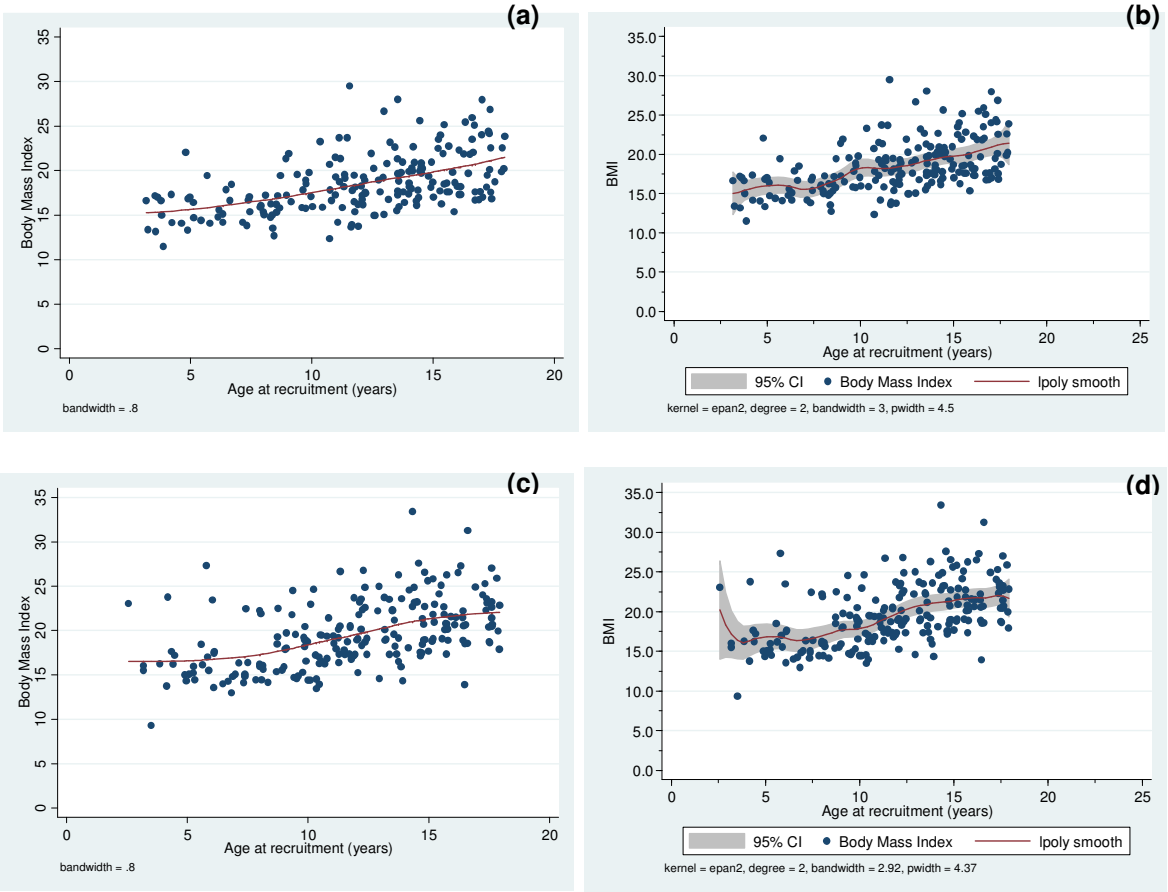
Outcome	Sex	Model	*n*	Coefficients			
				Parameter	Estimate	95% confidence interval	*p*-value
cholesterol							
				β_1	-2.49	(-19.93, 14.69)	0.775
				β_2	-7.18	(-22.93, 8.56)	0.369
	Female	$y_i = \alpha_i + \beta_{1i}x_i + \beta_{2i}ln(x_i)ln(x_i)$		α	116.04	(109.49, 122.60)	<0.001
				β_1	-9.54	(-25.28, 6.20)	0.233
				β_2	-19.08	(-46.06, 7.91)	0.165
High density lipoprotein (HDL) cholesterol	**Male**	$y_i = \alpha_i + {\beta_{1i}}/{x_i^2} + \beta_{2i}x_i^3$	163	α	50.66	(47.82, 53.51)	<0.001
				β_1	0.62	(-1.02, 2.25)	0.457
				β_2	-1.96	(-3.72, -0.19)	0.030
	Female	$y_i = \alpha_i + {\beta_{1i}}/{x_i^2} + \beta_{2i}x_i^3$	188	α	54.42	(51.73, 57.10)	<0.001
				β_1	0.45	(-1.09, 1.98)	0.568
				β_2	-0.62	(-2.22, 0.97)	0.440
Fasting triglyceride	**Male**	$y_i = \alpha_i + {\beta_{1i}}/{x_i^2} + \beta_{2i}x_i$	139	α	114.5	(98.60, 130.3)	<0.001
				β_1	15.03	(1.01, 29.0)	0.036

Outcome	Sex	Model	*n*	Coefficients			
				Parameter	Estimate	95% confidence interval	*p*-value
	Female	$y_i = \alpha_i + \beta_{1i}x_i^2 + \beta_{2i}x_i^3$	166	β_2	93.3	(33.3, 153.3)	0.003
				α	104.03	(90.30, 117.8)	<0.001
				β_1	94.17	(16.39, 171.94)	0.018
				β_2	-49.70	(-91.05, -8.34)	0.019
Fasting insulin	**Male**	$y_i = \alpha_i + \beta_{1i}x_i + \beta_{2i}x_i(ln(x_i))$	93	α	9.77	(7.57, 11.97)	<0.001
				β_1	8.60	(2.97, 14.23)	0.003
				β_2	10.49	(-0.32, 21.29)	0.057
	Female	$y_i = \alpha_i + {\beta_{1i}}/{x_i^2} + {\beta_{2i}(\ln(x_i))}/{x_i^2}$	103	α	12.29	(10.95, 13.63)	<0.001
				β_1	-12.23	(-16.75, -5.87)	<0.001
				β_2	-11.31	(-16.75, -5.87)	<0.001
Fasting glucose	**Male**	$y_i = \alpha_i + \beta_{1i}x_i^2 + \beta_i x_i^2(\ln(x_i))$	139	α	84.28	(82.09, 86.47)	<0.001
				β_1	13.55	(5.06, 22.04)	0.002
				β_2	-18.70	(-30.80, -6.59)	0.003
Fasting glucose	**Females**	$y_i = \alpha_i + \beta_{1i}x_i + \beta_{2i}x_i^2$	160	α	82.14	(80.44, 83.84)	<0.001

Outcome	Sex	Model	*n*	Coefficients			
				Parameter	Estimate	95% confidence interval	*p*-value
				β_1	26.73	(9.12, 44.34)	0.003
				β_2	-11.20	(-18.97, -3.44)	<0.001

C.5 Body mass index analysis

Figure C-1: Modelling body mass index as a function of age at recruitment in: males estimated by (a) locally weighted smoothing (b) fractional polynomial model: and, in females estimated by (c) locally weighted smoothing, and (d) fractional polynomial model

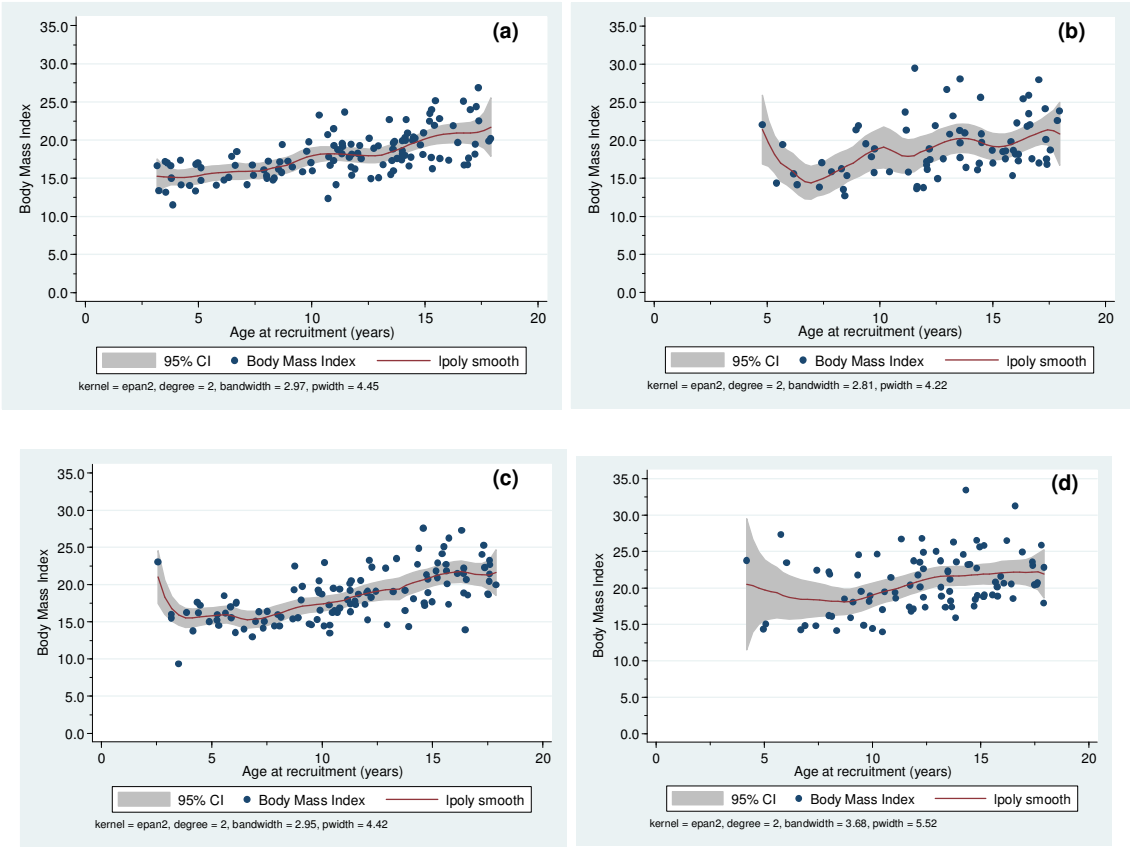


Both fractional polynomial models are of 2 orders, estimated over 44 models. Fractional polynomial model for males: $y_i = \alpha_i + \beta_{1i}\ln(x_i) + \beta_{2i}\sqrt{x_i}$: **(*n* = 195). Fractional polynomial model for females:** $y_i = \alpha_i + {}^{\beta_{1i}}/_{x_i^2} + \beta_{2i} ln(x_i)$ **: (*n* = 213). Shaded area in (b) and (d) denote 95% confidence interval.**

Table C-5: Fractional polynomial models fitting body mass index as a function of age at recruitment, stratified by sex and lipodystrophy syndrome status

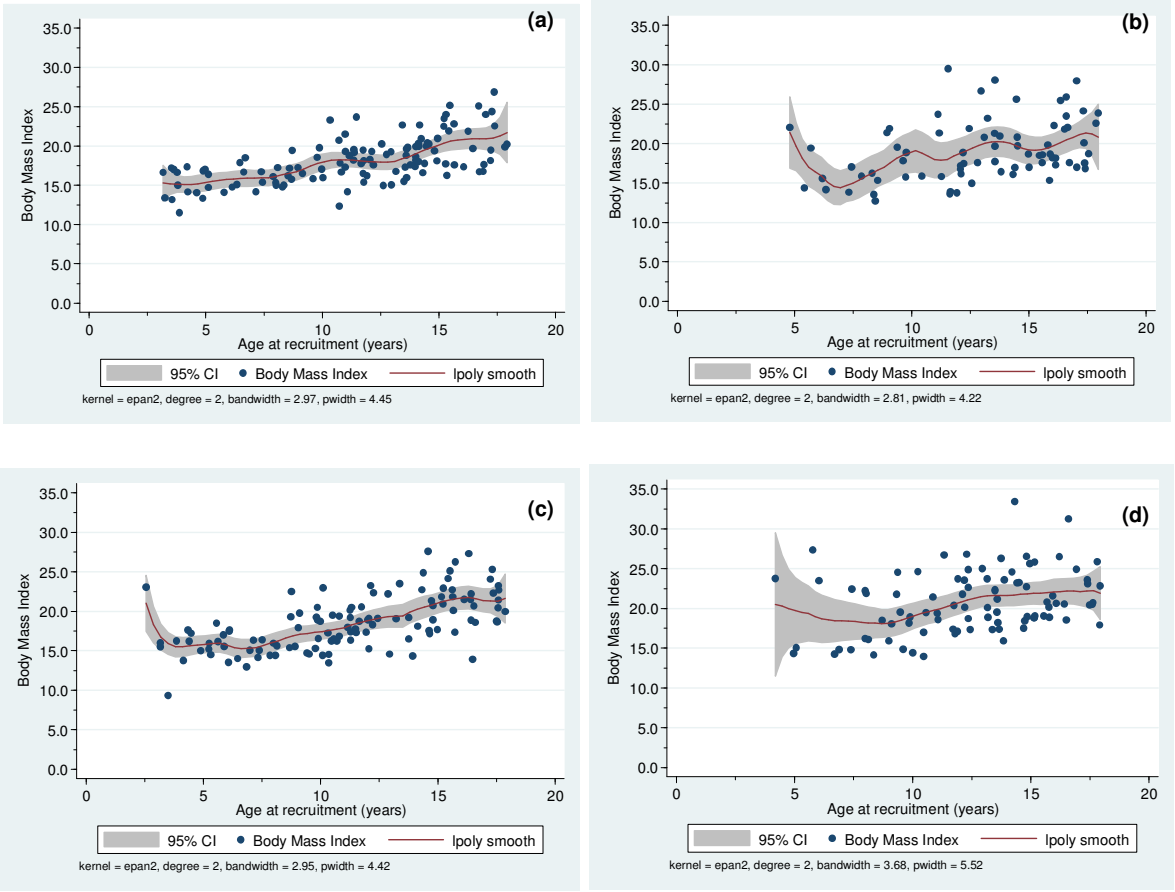
Outcome	Sex	Model	*n*	Coefficients			
				Parameter	Estimate	95% confidence interval	*p*-value
No lipodystrophy	**Male**	$y_i = \alpha_i + \beta_{1i}\sqrt{x_1} + \beta_{2i}x_i^3$	86	α	18.35	17.65, 19.05	<0.001
				β_1	3.16	-0.23, 12.34	0.051
				β_2	0.35	-0.42, 1.12	0.368
	Female	$y_i = \alpha_i + {\beta_{1i}}/{x_i^2} + \beta_{2i}\ln(x_i)$	89	α	18.86	18.25, 19.46	<0.001
				β_1	1.09	0.59, 1.59	<0.001
				β_2	8.10	5.65, 10.56)	<0.001
Lipodystrophy	**Male**	$y_i = \alpha_i + \beta_{1i}x_i^3 + \beta_{2i}x_i^3(ln(x_i))$	106	α	18.47	17.56, 19.39	<0.001
				β_1	2.21	0.40, 4.02	0.017
				β_2	-2.01	-4.99, 0.97	0.184
	Female	$y_i = \alpha_i + \beta_{1i}x_i^3 + \beta_{2i}x_i^3(ln(x_i))$	119	α	19.92	19.03, 20.78	<0.001
				β_1	3.52	1.55, 5.48	0.001
				β_2	-3.91	-7.14, -0.67	0.018

Figure C-2: Relationship between age and body mass index (m/kg^2) in: (a) males without lipodystrophy (*n* = 86), (b) males with lipodystrophy (*n* = 106), (c) females without lipodystrophy (*n* =89), and (d) female with lipodystrophy (*n* =119)



Equations: (a) $y_i = \alpha_i + \beta_{1i}\sqrt{x_1} + \beta_{2i}x_i^3$ **(b)** $y_i = \alpha_i + \beta_{1i}x_i^3 + \beta_{2i}x_i^3(ln(x_i))$ **(c)** $y_i = \alpha_i + {}^{\beta_{1i}}\!/_{x_i^2} + \beta_{2i}\ln(x_i)$**nd (d)** $y_i = \alpha_i + \beta_{1i}x_i^3 + \beta_{2i}x_i^3\big(ln(x_i)\big)$ **Fractional polynomial models were of 2 orders, estimated over 44 models. Shaded area denotes 95% confidence interval. S*ee* Table C5 for details of models.**

Figure C-3: Modelling body mass index (m/kg^2) as a function of age at recruitment in: (a) males without body fat disorder (*n* = 120), (b) males with body fat disorder (*n* = 75), (c) females without body fat disorder (*n* = 119), and (d) female with body fat disorder (*n* = 92)



Equations: **(a)** $y_1 = \alpha_i + \beta_{1i}(ln(x_i) + \beta_{2i}x_i$ **(b)** $y_i = \alpha_i + {\beta_{1i}}/{x_i^2} + {\beta_{2i}(ln(x_i))}/{x_i^2}$ **(c)** $y_i = \alpha_i + {\beta_{1i}}/{x_i} + \beta_{2i}(ln(x_i))$ **and (d)** $y_i = \alpha_i + {\beta_{1i}}/{x_i^2} = {\beta_{2i}(ln(x_i))}/{x_i^2}$

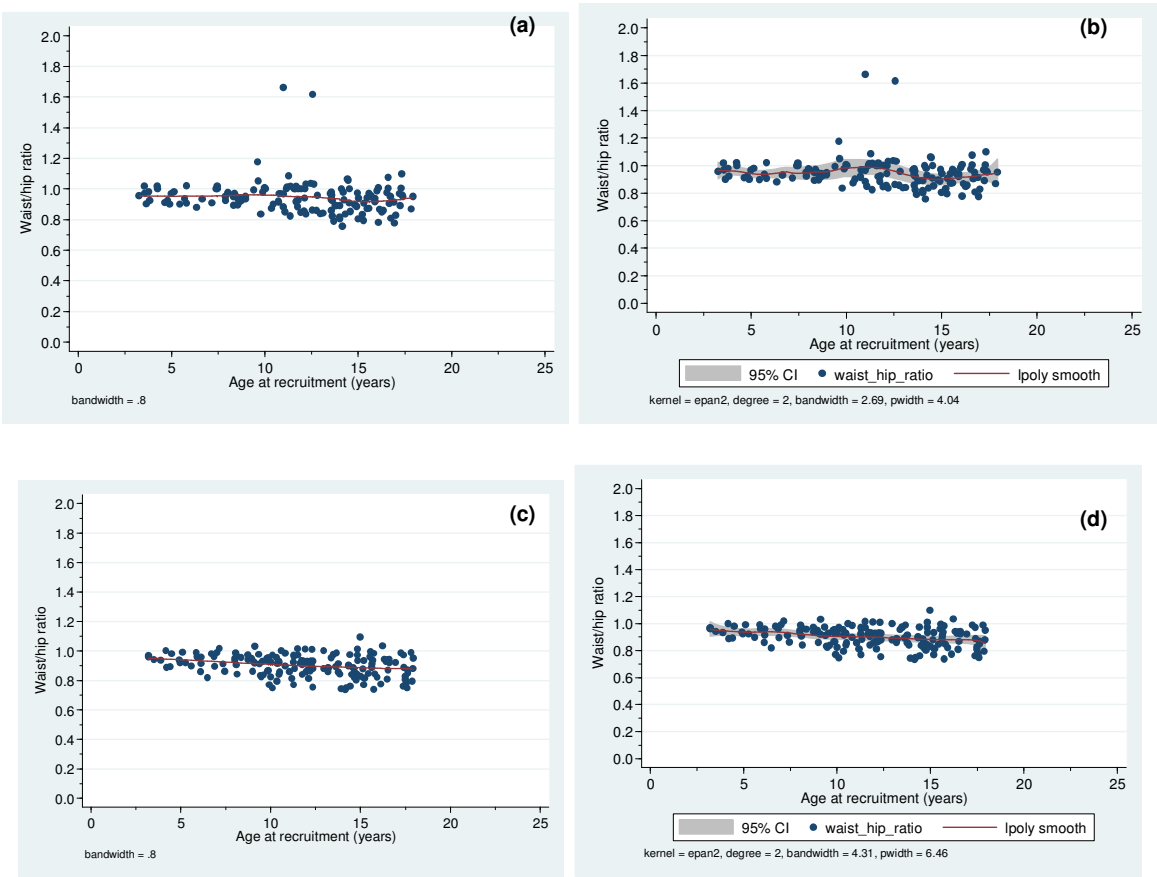
Fractional polynomial models are of 2 orders, estimated over 44 models. Shaded area denotes 95% confidence interval. See Table C-6 for details of model.

Table C-6: Fractional polynomial models fitting body mass index as a function of age at recruitment, stratified by sex and body fat disorder status

Outcome	Sex	Model	*n*	Coefficients			
				Parameter	Estimate	95% confidence interval	*p*-value
No fat disorder	**Male**	$y_1 = \alpha_i + \beta_{1i}(ln(x_i) + \beta_{2i}x_i$	120	α	17.93	17.38, 18.48	<0.001
				β_1	-2.22	-6.45, 2.00	0.300
				β_2	6.88	2.21, 11.56	0.004
	Female	$y_i = \alpha_i + {}^{\beta_{1i}}/_{x_i} + \beta_{2i}(ln(x_i))$	119	α	18.45	17.94, 18.97	<0.001
				β_1	1.02	0.59, 1.45	<0.001
				β_2	8.49	6.39, 10.59	<0.001
Fat disorder	**Male**	$y_i = \alpha_i + {}^{\beta_{1i}}/_{x_i^2} = {}^{\beta_{2i}(ln(x_i))}/_{x_i^2}$	75	α	19.18	18.32, 20.04	<0.001
				β_1	-7.57	-11.92, -3.22)	0.001
				β_2	-8.76	-14.65, -2.88	0.004
	Female	$y_i = \alpha_i + {}^{\beta_{1i}}/_{x_i^2} = {}^{\beta_{2i}(ln(x_i))}/_{x_i^2}$	92	α	20.81	20.01, 21.61	<0.001
				β_1	-6.14	-9.67, -2.62	0.001
				β_2	-6.45	-10.66, -2.24	0.003

C.6 Waist/hip ratio

Figure C-4: Modelling waist/hip ratio as a function of age at recruitment in: males estimated by (a) locally weighted smoothing, (b) fractional polynomial model: and, in females estimated by (c) locally weighted smoothing, and (d) fractional polynomial model

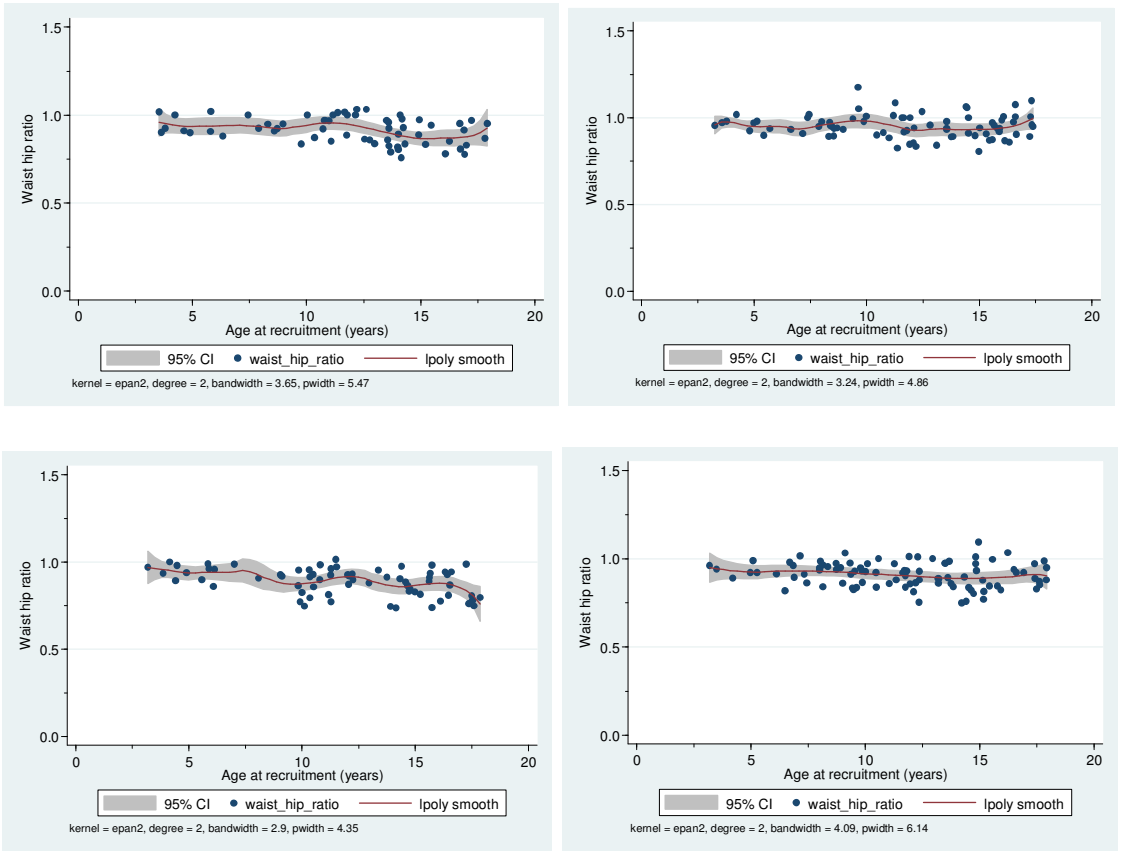


Both fractional polynomial models are of 2 orders, estimated over 44 models. Fractional polynomial for males: $y_i = \alpha_i + \beta_{1i}x_i + \beta_{2i}x_i$.: (*n* = 146). Fractional polynomial model for females:$y_i = \alpha_i + \beta_{1i}x_i + \beta_{2i}x_i^2$: : (*n* = 164). Shaded area in (b) and (d) denote 95% confidence interval.

Table C-7: Fractional polynomial models fitting waist/hip ratio as a function of age at recruitment, stratified by sex and lipodystrophy syndrome status

Outcome	Sex	Model	*n*	Coefficients			
				Parameter	Estimate	95% confidence interval	*p*-value
No lipodystrophy	**Male**	$y_i = \alpha_i + \beta_{1i}x_i + \beta_{2i}x_i(ln(x_i))$	63	α	0.94	0.91, 0.98	<0.001
				β_1	0.19	-0.18, 0.56	0.308
				β_2	-0.26	-0.62, 0.10	0.158
	Female	$y_i = \alpha_i + {}^{\beta_{1i}}/_{x_i} + \beta_{2i}x_i^3$	68	α	0.89	0.87, 0.91	<0.001
				β_1	0.01	-0.02, 0.07	0.136
				β_2	-0.01	-0.03, 0.00	0.136
Lipodystrophy	**Male**	$y_i = \alpha_i + \beta_{1i}x_i + \beta_{2i}x_i(ln(x_i))$	80	α	0.96	0.93, 0.99	<0.001
				β_1	0.02	-0.29, 0.32	0.907
				β_2	-0.03	-0.31, 0.26	0.861
	Female	$y_i = \alpha_i + \beta_{1i}x_i^3 + \beta_{2i}x_i^3(ln(x_i))$	94	α	0.90	0.88, 0.92	<0.001
				β_1	-0.04	-0.08, 0.00	0.080
				β_2	0.05	-0.02, 0.12	0.128

Figure C-5: Relationship between age and waist/hip ratio in: (a) males without lipodystrophy (*n* = 63), (b) males with lipodystrophy (*n* = 80), (c) females without lipodystrophy (*n* = 68), and (d) female with *lipodystrophy (n =* 94*)*



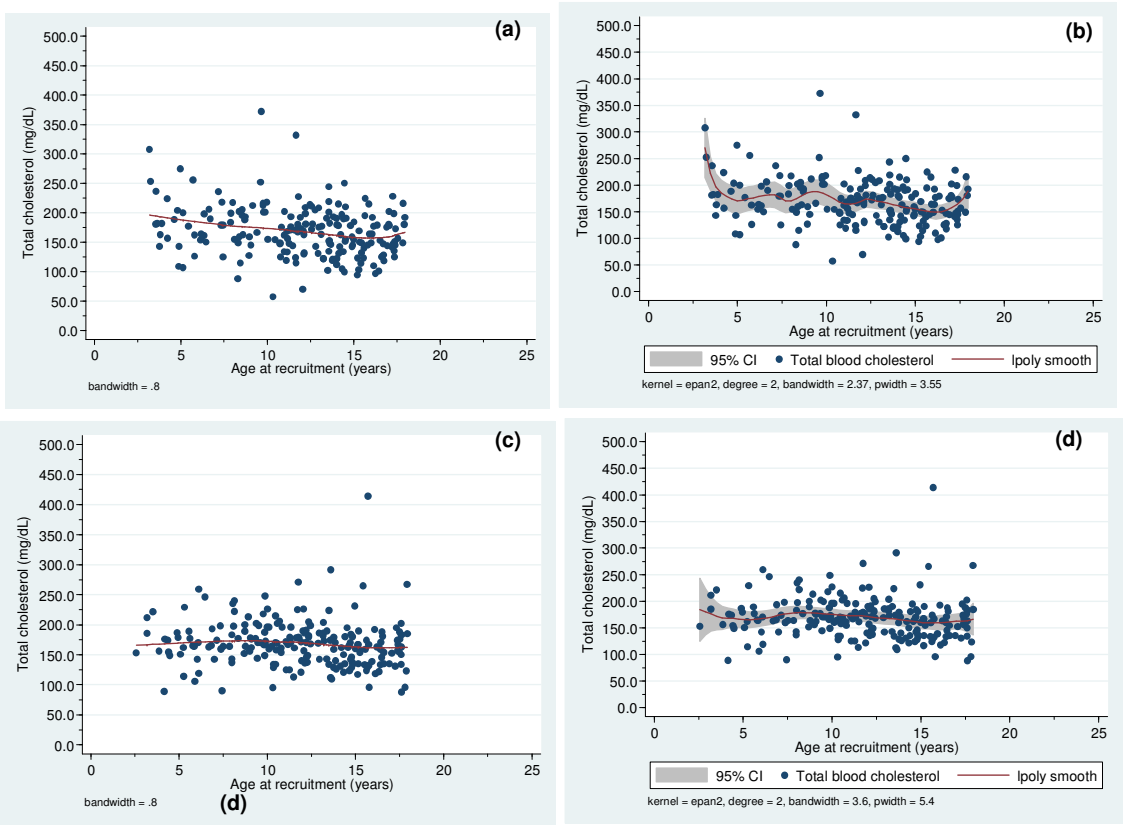
Equations: (a) $y_i = \alpha_i + \beta_{1i}x_i + \beta_{2i}x_i(\ln(x_i))$ **(b)** $y_i = \alpha_i + \beta_{1i}x_i + \beta_{2i}x_i(\ln(x_i))$**(c)**$y_i = \alpha_i + {}^{\beta_{1i}}/_{x_i} + \beta_{2i}x_i^3$ **and (d)**$y_i = \alpha_i + \beta_{1i}x_i^3 + \beta_{2i}x_i^3(\ln(x_i))$**. Fractional polynomial models were of 2 orders, estimated over 44 models. Shaded area denotes 95% confidence interval. See Table C-7 for details of model.**

Table C-8: Fractional polynomial models fitting waist/hip ratio as a function of age at recruitment, stratified by sex and body fat disorder status

Outcome	Sex	Model	*n*	Coefficients			
				Parameter	Estimate	95% confidence interval	*p*-value
No fat disorder	**Male**	$y_i = \alpha_i + \beta_{1i}x_i + \beta_{2i}x_i(ln(x_i))$	93	α	0.95	0.92, 0.97	<0.001
				β_1	0.09	-0.17, 0.34	0.490
				β_2	-0.16	-0.41, 0.09	<0.001
	Female	$y_i = \alpha_i + {}^{\beta_{1i}}/_{x_i} + \beta_{2i}x_1$	93	α	0.90	0.88, 0.92	<0.001
				β_1	0.01	0.00, 0.01	0.225
				β_2	-0.01	-0.02, 0.00	0.010
Fat disorder	**Male**	$y_i = \alpha_i + \beta_{1i}(ln(x_i)) + \beta_{2i}((ln\, x_i)^2)$	53	α	0.97	0.93, 1.01	<0.001
				β_1	0.01	-0.09, 0.11	0.808
				β_2	-0.12	-0.39, 0.15	0.395
	Female	$y_i = \alpha_i + \beta_{1i}x_i^3 + \beta_{2i}x_i^3(ln(x_i))$	71	α	0.90	0.88, 0.93	<0.001
				β_1	-0.04	-0.10, 0.02	0.150
				β_2	0.06	-0.03, 0.15	0.192

C.7 Total cholesterol

Figure C-6: Modelling total cholesterol as a function of age at recruitment: in males estimated by (a) locally weighted smoothing and (b) fractional polynomial model: and, in females estimated by (c) locally weighted smoothing, and (d) fractional polynomial model

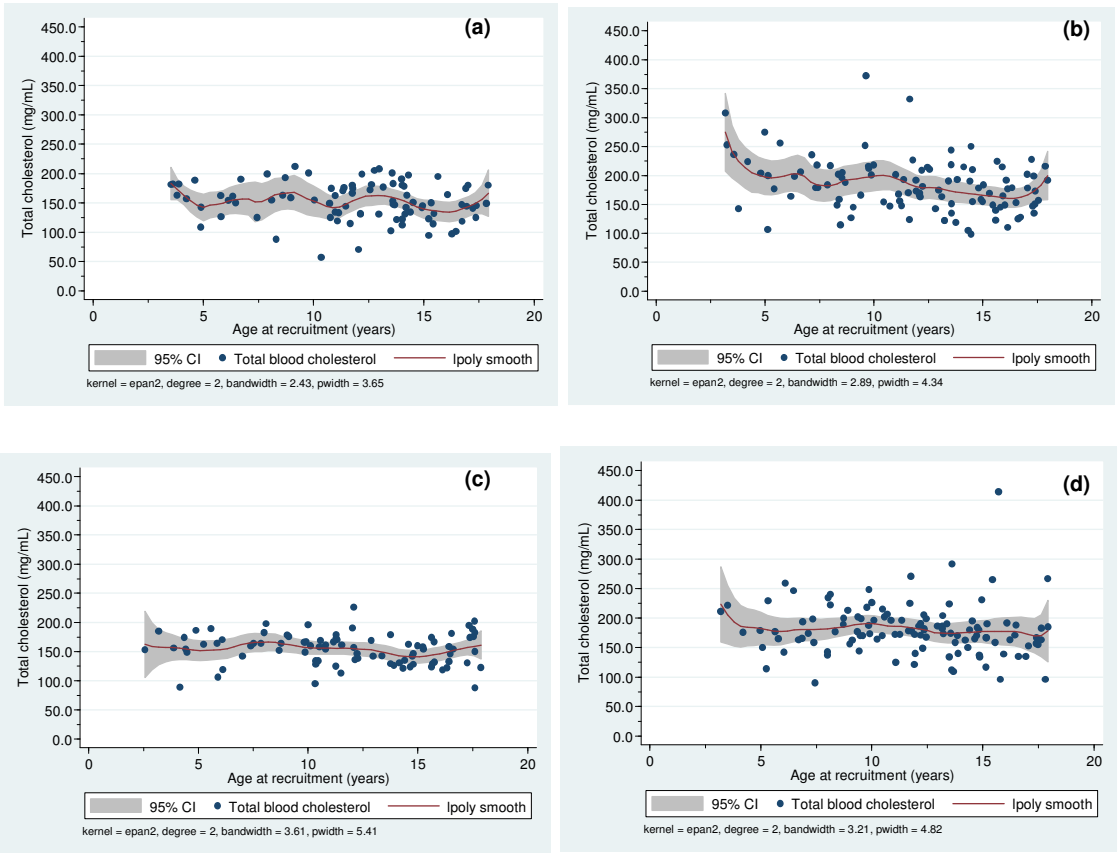


Fractional polynomial models are of 2 orders, estimated over 44 models. Fractional polynomial model or males: $y_i = \alpha_i + {\beta_{1i}}/{x_i^2} + \beta_{2i}x_i^2$ (n = 197). Fractional polynomial model for females: $y_i = \alpha_i + \beta_{1i}x_i + \beta_{2i}x_i ln(x_i)$:: ($n$ = 211). Shaded area in (b) and (d) denote 95% confidence interval.

Table C-9: Fractional polynomial models fitting total cholesterol as a function of age at recruitment, stratified by sex and lipodystrophy syndrome status

Outcome	Sex	Model	*n*	Coefficients			
				Parameter	Estimate	95% confidence interval	*p*-value
No lipodystrophy	**Male**	$y_i = \alpha_i + {\beta_{1i}}/{x_i^2} + \beta_{21}x_i^3$	88	α	151.89	143.29, 160.48	<0.001
				β_1	2.15	-3.14, 7.45	0.553
				β_2	-1.68	-7.28, 3.93	<0.001
	Female	$y_i = \alpha_i + \beta_{1i}x_i^3 + \beta_{2i}x_i^3(ln(x_i))$	89	α	151.93	143.85, 160.00	<0.001
				β_1	-8.56	-24.02, 6.91	0.274
				β_2	12.05	-13.32, 37.42	0.348
Lipodystrophy	**Male**	$y_i = \alpha_i + {\beta_{1i}}/{x_i^2} + \beta_{2i}x_i^3$	107	α	180.5	170.2, 190.7	<0.001
				β_1	6.77	0.89, 12.66	0.025
				β_2	-3.76	-9.87, 2.35	0.225
	Female	$y_i = \alpha_i + {\beta_{1i}}/{x_i^2} + \beta_{2i}x_i^3$	119	α	180.84	171.57, 190.10	<0.001
				β_1	1.27	-5.63, 8.17	0.716
				β_2	-2.34	-8.39, 3.69	0.443

Figure C-7: Relationship between age and total cholesterol in: (a) males without lipodystrophy syndrome (*n* = 88), (b) males with lipodystrophy syndrome (*n* = 107), (c) females without lipodystrophy syndrome (*n* =89), and (d) female with lipodystrophy syndrome (*n* =119)

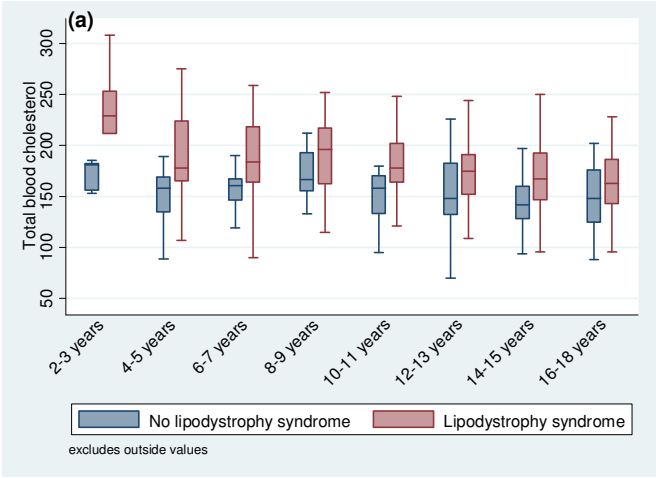


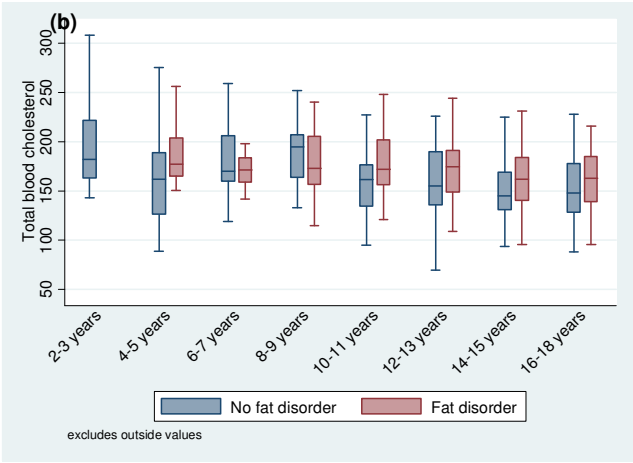
Equations: **(a)** $y_i = \alpha_i + {}^{\beta_{1i}}\!/_{x_i^2} + \beta_{21}x_i^3$ **(b)** $y_i = \alpha_i + {}^{\beta_{1i}}\!/_{x_i^2} + \beta_{2i}x_i^3$ **(c)** $y_i = \alpha_i + \beta_{1i}x_i^3 + \beta_{2i}x_i^3(ln(x_i))$ **and (d)** $y_i = \alpha_i + {}^{\beta_{1i}}\!/_{x_i^2} + \beta_{2i}x_i^3$ **Fractional polynomial models were of 2 orders, estimated over 44 models. Shaded area denotes 95% confidence interval. S*ee* appendix for details of models. See Table C-9 for details of models.**

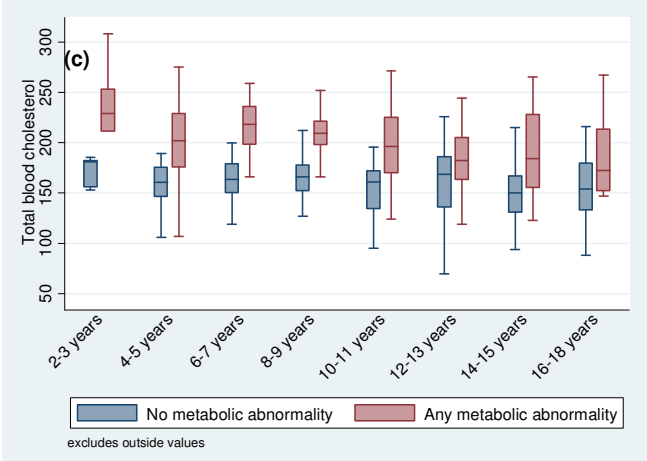
Table C-10: Fractional polynomial models fitting total cholesterol as a function of age at recruitment, stratified by sex and metabolic abnormality status

Outcome	Sex	Model	*n*	Coefficients			
				Parameter	Estimate	95% confidence interval	*p*-value
No metabolic abnormality	**Male**	$y_i = \alpha_i + \beta_{1i}x_i^3 + \beta_{2i}x_i^3(ln(x_i))$	141	α	156.24	148.64, 163.83	<0.001
				β_1	-7.95	-23.99, 8.09	0.329
				β_2	10.21	-15.89, 36.30	0.441
	Female	$y_i = \alpha_i + \beta_{1i}x_i + \beta_{2i}x_i^2$	159	α	160.26	153.02, 167.51	<0.001
				β_1	21.31	-54.01, 96.64	0.577
				β_2	-9.82	-42.70, 23.06	0.556
Metabolic abnormality	**Male**	$y_i = \alpha_i + {}^{\beta_{1i}}/_{x_i} + \beta_{2i}x_i^3$	54	α	197.71	179.75, 215.67	<0.001
				β_1	4.44	-3.14, 12.03	0.245
				β_2	-5.72	-16.53, 5.10	0.294
	Female	$y_i = \alpha_i + \beta_{1i}x_i^3 + \beta_{2i}x_i^3(ln(x_i))$	49	α	199.33	185.41, 213.26	<0.001
				β_1	-6.93	-39.72, 25.85	0.672
				β_2	12.70	-44.33, 69.74	0.656

Figure C-8: Comparison of median total cholesterol across age groups between subjects with/without (a) lipodystrophy syndrome (*n* = 403), (b) fat disorder (*n* = 407), and (c) metabolic abnormality (*n* = 403)





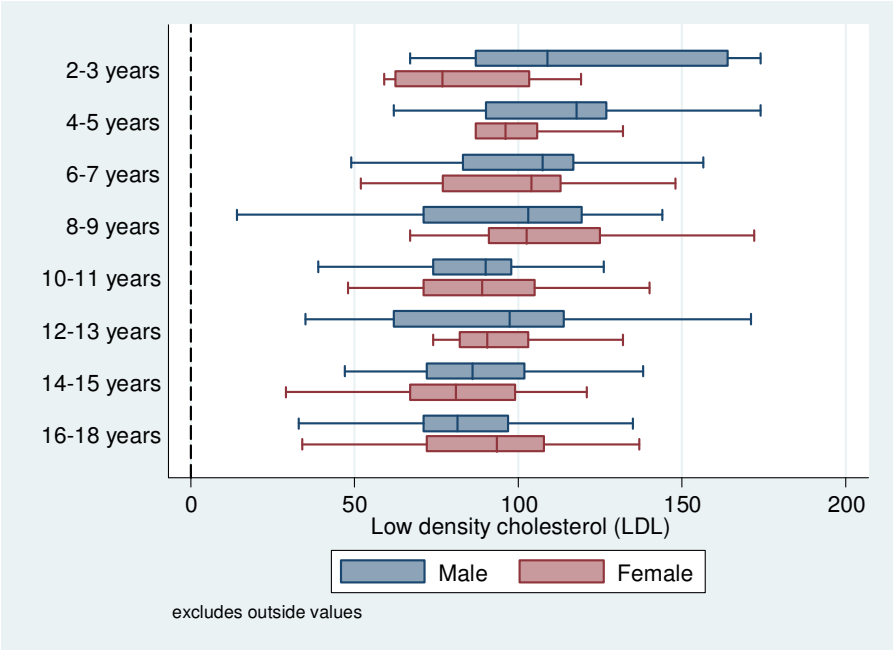


Bars indicate 25th and 75th percentiles of the distribution within each age band. No significant differences were seen in median total cholesterol between subjects with fat disorder and those without ($p > 0.05$)

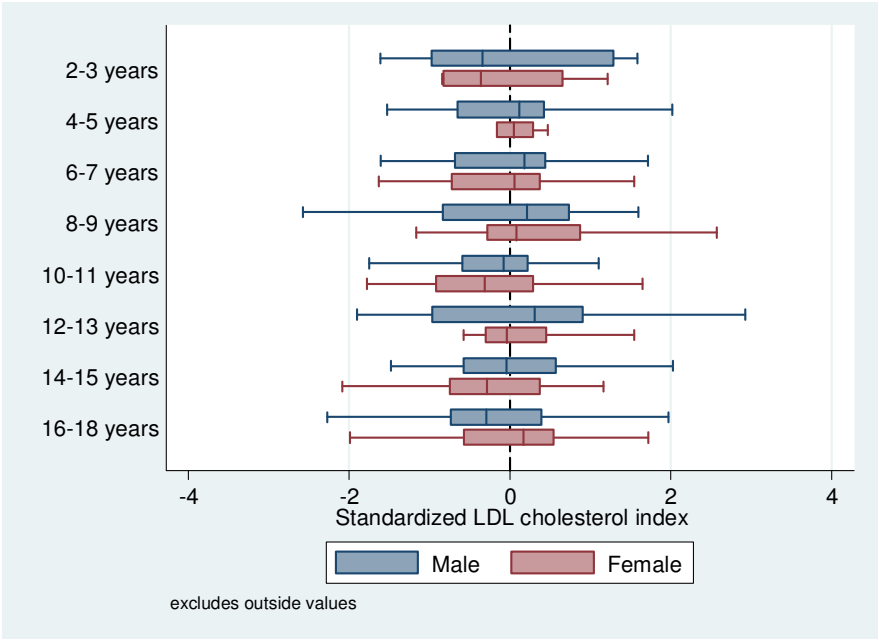
C.8 Low density lipoprotein cholesterol

Figure C-9: Low density lipoprotein cholesterol across age groups: (a) median LDL cholesterol, and (b) median standardized LDL cholesterol

(a)

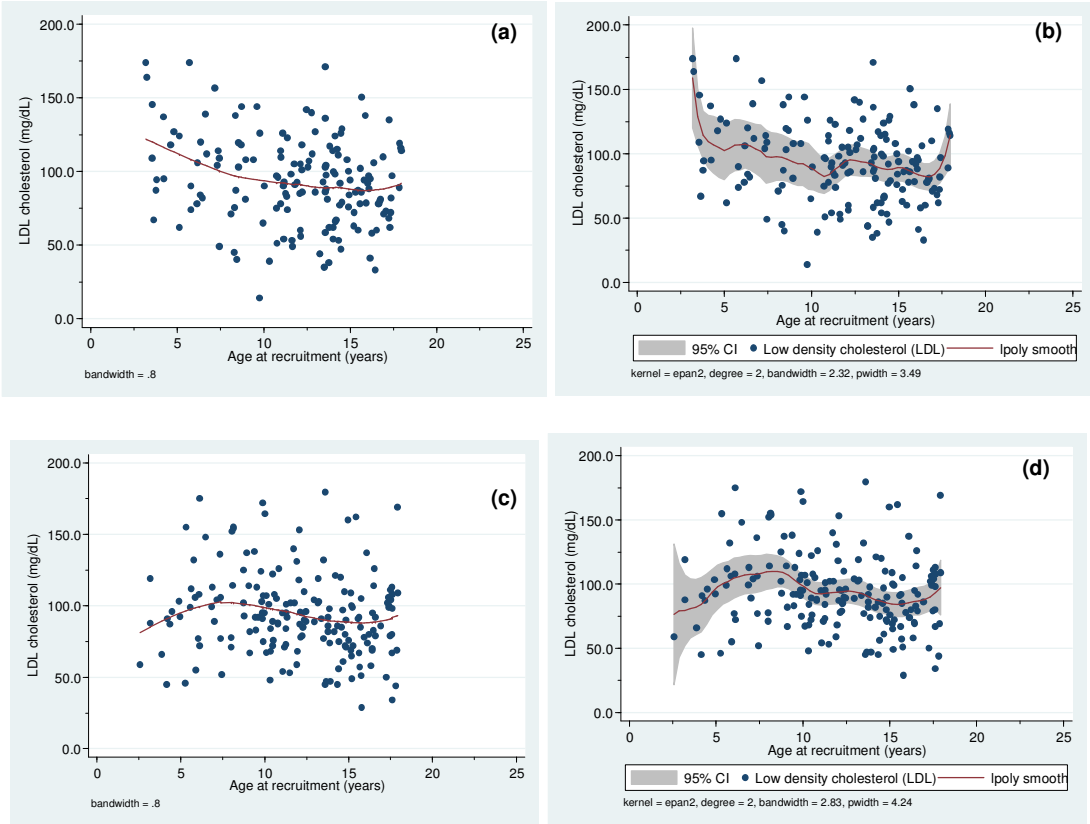


(b)



Boxes demark 25th and 75th percentiles (2nd and 3rd quartile) and lines indicate adjacent values (most extreme values within 1.5 interquartile range of the nearest quartile) of the distribution within each age band. Values outside adjacent values excluded for clarity. *n* =349 for LDL cholesterol, and *n* = 341 for standardized LDL cholesterol. Comparison of median standardized LDL-cholesterol between males and females: $p > 0.05$ for all groups.

Figure C-10: Modelling LDL cholesterol as a function of age at recruitment in: males estimated by (a) locally weighted smoothing, (b) fractional polynomial model: and, in females estimated by (c) locally weighted smoothing, and (d) fractional polynomial model.

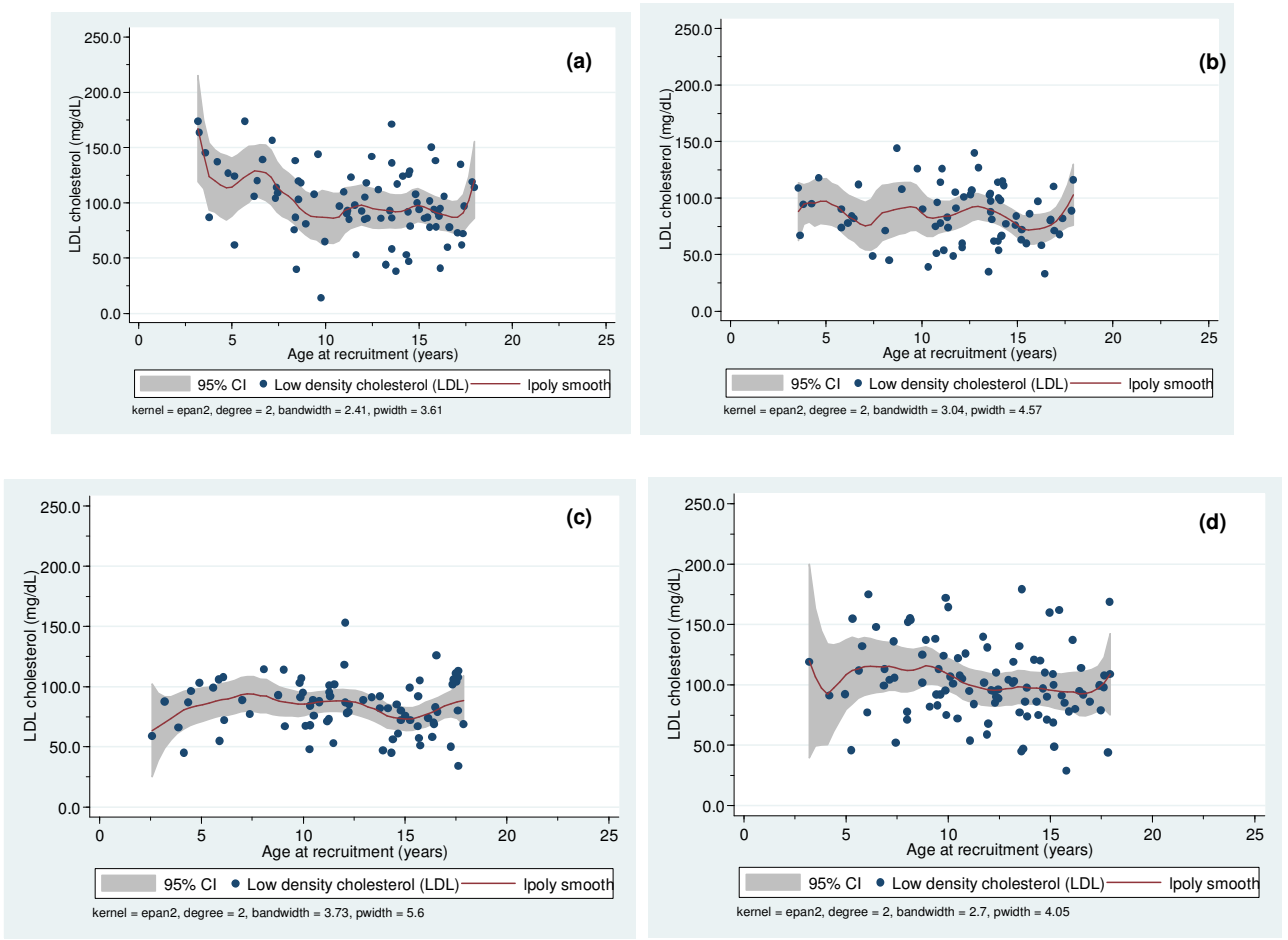


Both fractional polynomial models are of 2 orders, estimated over 44 models. Fractional polynomial model for males: $y_i = \alpha_i + {}^{\beta_{1i}}/_{x_i^2} + \beta_{2i}x_i$**: : (*n* = 158). Fractional polynomial model for females:** $y_i = \alpha_i + {}^{\beta_{1i}}/_{x_i^2} + \beta_{2i}x_i$**: (*n* = 183). Shaded area in (b) and (d) denote 95% confidence interval.**

Table C-11: Fractional polynomial models fitting LDL-cholesterol as a function of age at recruitment, stratified by sex and lipodystrophy syndrome status

Outcome	Sex	Model	*n*	Coefficients			
				Parameter	Estimate	95% confidence interval	*p*-value
No lipodystrophy syndrome	**Male**	$y_i = \alpha_i + {}^{\beta_{1i}}/_{x_i^2} + \beta_{2i}x_i^3$	72	α	85.03	77.74, 92.32	<0.001
				β_1	0.97	-3.65, 5.58	0.678
				β_2	-0.97	-5.82, 3.88	0.692
	Female	$y_i = \alpha_i + {}^{\beta_{1i}}/_{x_i} + {}^{\beta_{2i}}/_{\sqrt[2]{x_i}}$	80	α	84.78	79.62, 89.94	<0.001
				β_1	-46.78	-101.77, 8.22	0.094
				β_2	111.34	-22.52, 245.20	0.102
Lipodystrophy syndrome	**Male**	$y_i = \alpha_i + {}^{\beta_{1i}}/_{x_i} + \beta_{2i}x_i^3$	86	α	93.97	85.29, 102.65	<0.001
				β_1	27.50	10.53, 44.47	0.002
				β_2	1.90	-3.94, 7.73	0.520
	Female	$y_i = \alpha_i + \beta_{1i}x_i^3 + \beta_{2i}x_i^3(ln(x_i))$.	101	α	100.72	92.16, 109.27	<0.001
				β_1	-15.00	-34.01, 4.00	0.120
				β_2	19.41	-11.53, 50.34	0.216

Figure C-11: Modelling LDL cholesterol as a function of age at recruitment in: (a) males without lipodystrophy syndrome (*n* = 72), (b) males with lipodystrophy syndrome (*n* = 86), (c) females without lipodystrophy syndrome (*n* = 80), and (d) female with lipodystrophy syndrome (*n* = 101)

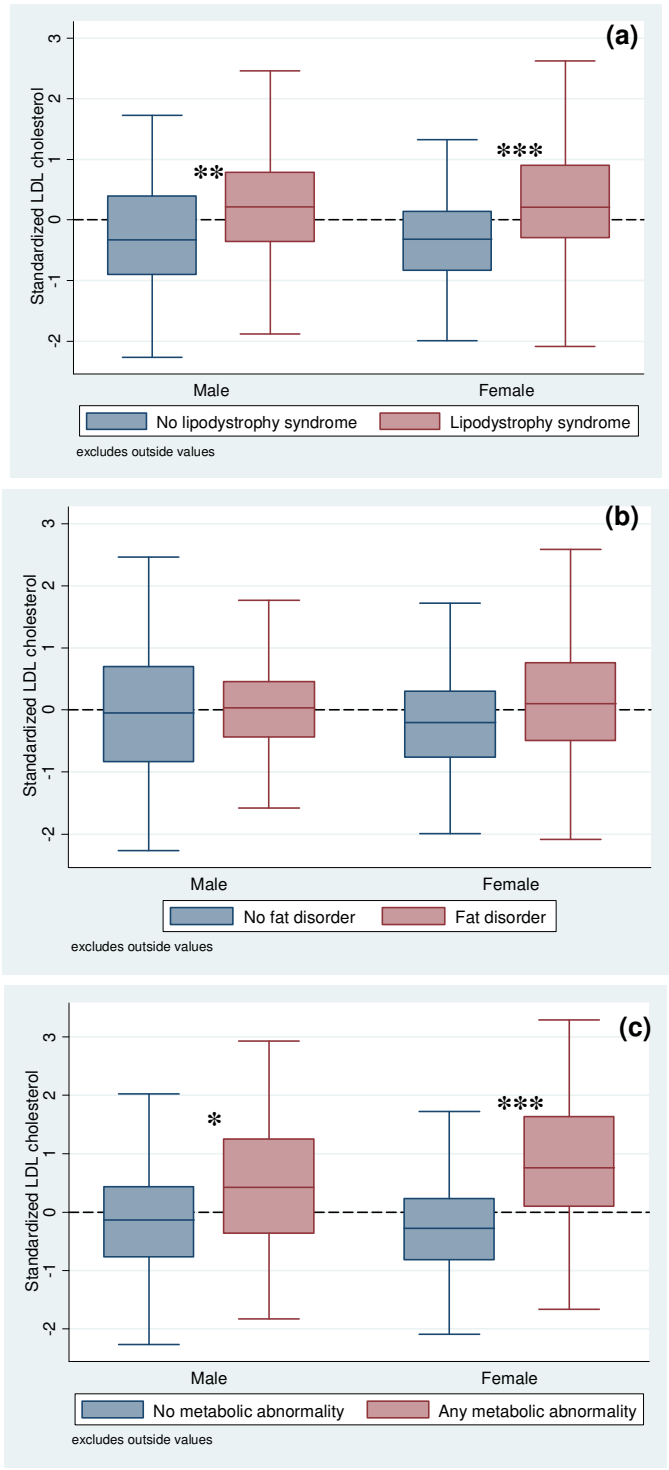


Equations: (a) $y_i = \alpha_i + {\beta_{1i}}/{x_i^2} + \beta_{2i}x_i^3$ **(b)** $y_i = \alpha_i + {\beta_{1i}}/{x_i} + \beta_{2i}x_i^3$ **(c)** $y_i = \alpha_i + {\beta_{1i}}/{x_i} + {\beta_{2i}}/{\sqrt[2]{x_i}}$ **and (d)** $y_i = \alpha_i + \beta_{1i}x_i^3 + \beta_{2i}x_i^3(ln(x_i))$. **Fractional polynomial of two orders, estimated over 44 models. Shaded area denotes 95% confidence interval. See Table C-11 for details of models.**

Table C-12: Fractional polynomial models fitting LDL-cholesterol as a function of age at recruitment, stratified by sex and metabolic abnormality status

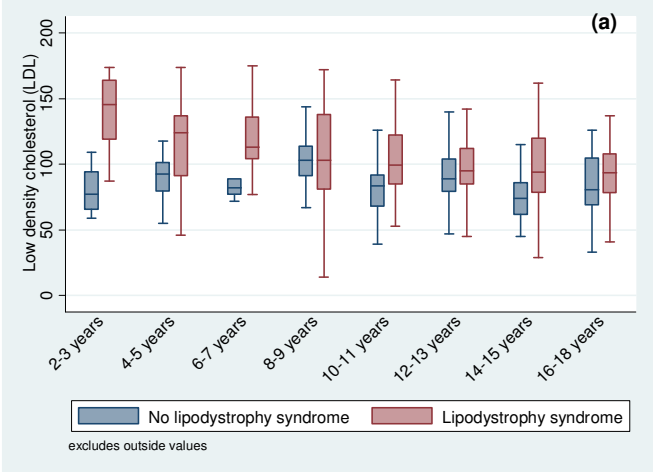
Outcome	Sex	Model	*n*	Coefficients			
				Parameter	Estimate	95% confidence interval	*p*-value
No metabolic abnormality	**Male**	$y_i = \alpha_i + \beta_{1i}\sqrt[2]{x_i} + \beta_{2i}x_i^3$	116	α	86.44	80.03, 92.84	<0.001
				β_1	-23.68	-89.61. 42.26	0.591
				β_2	2.04	-5.45, 9.52	0.591
	Female	$y_i = \alpha_i + {\beta_{1i}}/{x_i^2} + {\beta_{21}}/{x_i}$	136	α	85.36	81.44, 89.27	<0.001
				β_1	-5.52	-12.69, 1.65	0.130
				β_2	17.37	-7.60, 42.35	0.171
Metabolic abnormality	**Male**	$y_i = \alpha_i + {\beta_{1i}}/{x_i^2} + \beta_{2i}x_i$	42	α	103.30	90.01, 116.59	<0.001
				β_1	4.21	-3.14, 11.56	0.254
				β_2	-16.62	-59.14, 25.90	0.434
	Female	$y_i = \alpha_i + \beta_{1i}x_i^3 + \beta_{2i}x_i^3(ln(x_i)$	45	α	118.43	106.79, 130.07	<0.001
				β_1	-9.78	-38.13, 18.57	0.490
				β_2	18.61	-30.07, -67.29	0.445

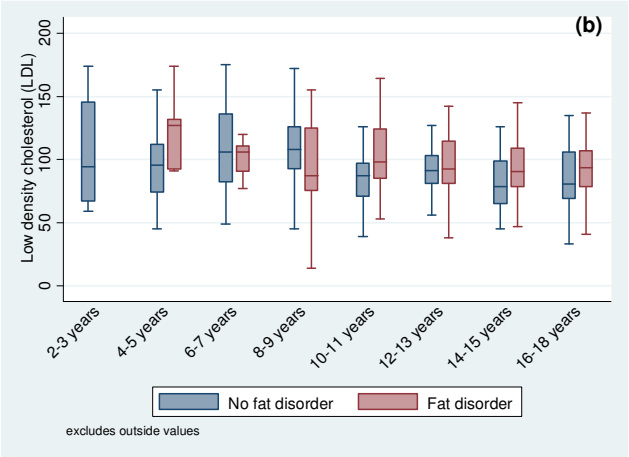
Figure C-12: Comparison of median standardized LDL-cholesterol, stratified by sex, between subjects with/without (a) lipodystrophy syndrome, (b) fat disorder, and (c) metabolic abnormality

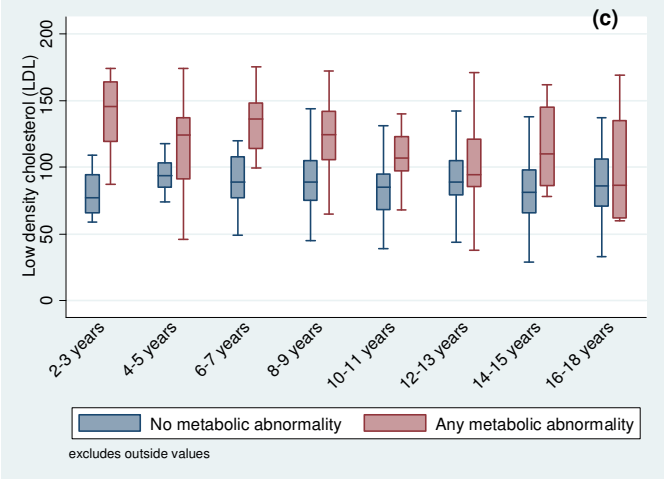


Boxes demark 25th and 75th percentiles (2nd and 3rd quartile) and lines indicate adjacent values (most extreme values within 1.5 interquartile range of the nearest quartile) of the distribution within each age band. Values outside adjacent values excluded for clarity. Comparison of median standardized LDL-cholesterol ratio between subjects with outcome and without outcome: *$p < 0.01$, **$p < 0.01$, ***$p < 0.001$

Figure C-13: Comparison of median low density lipoprotein (LDL) cholesterol across age groups between subjects with/without (a) lipodystrophy syndrome (n = 339), (b) fat disorder (n = 340), and (c) metabolic abnormality (n = 339)



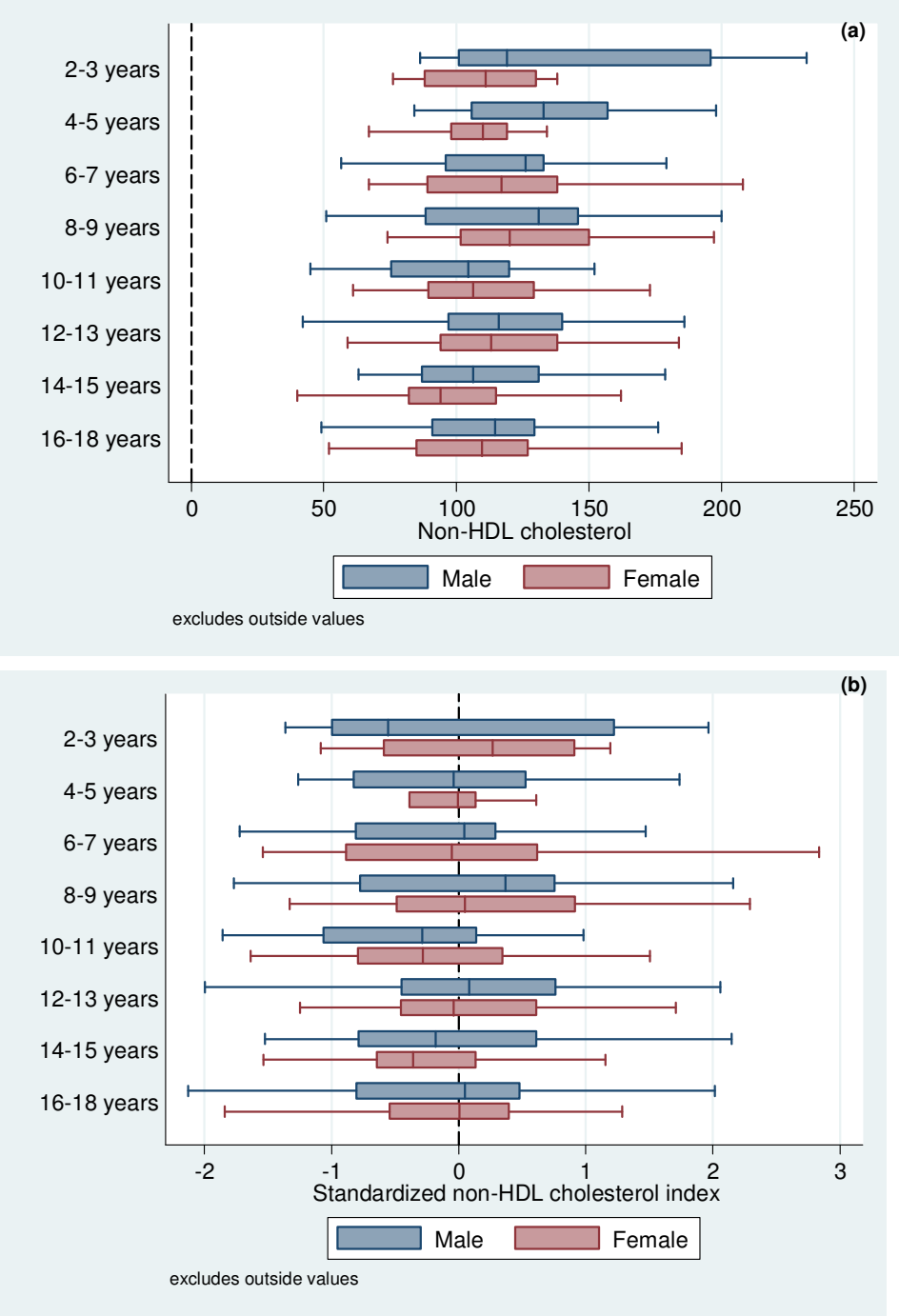




Bars indicate 25th and 75th percentile of the distribution within each age band. No significant differences were seen in median LDL cholesterol between subjects with fat disorder and those without ($p > 0.05$)

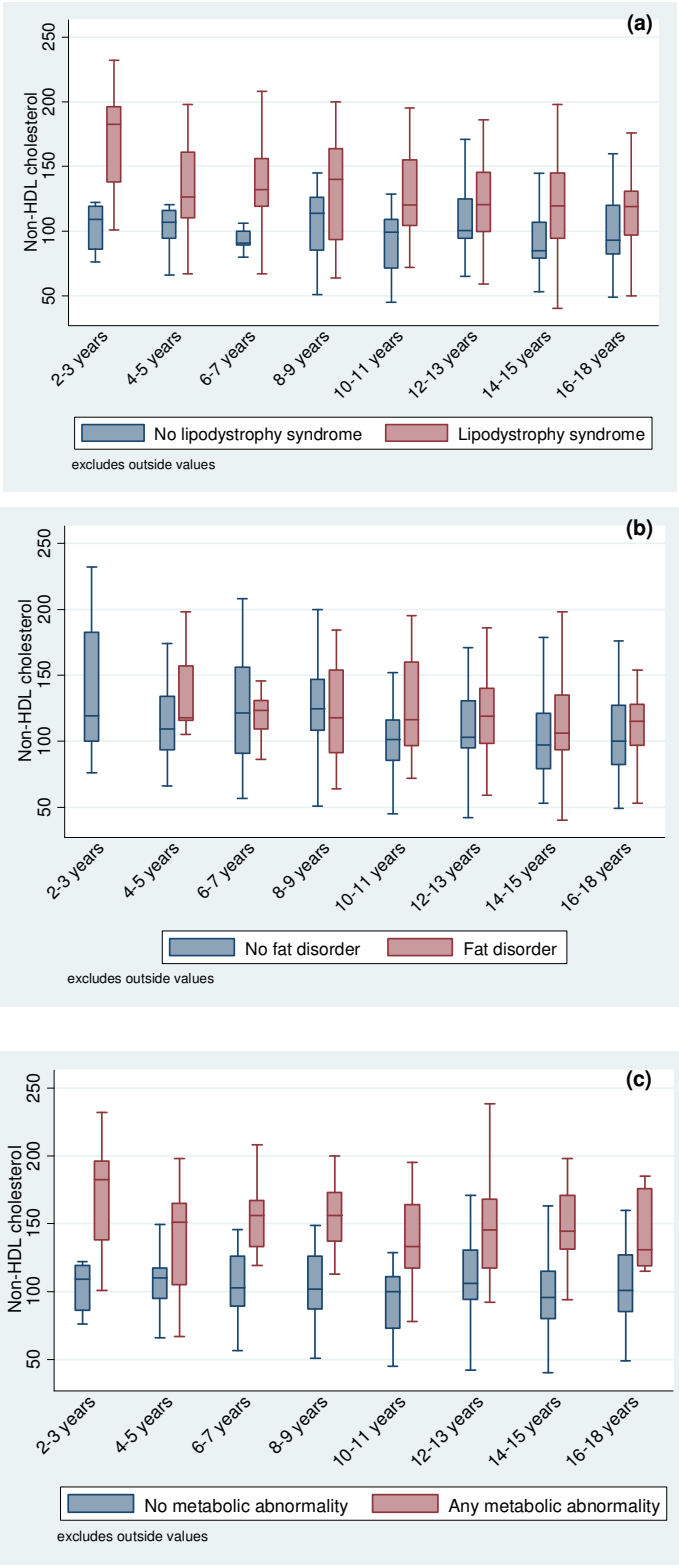
C.9 Non-high density lipoprotein cholesterol

Figure C-14: Non high density lipoprotein cholesterol across age groups: (a) median non-HDL cholesterol, and (b) median standardized non-HDL cholesterol



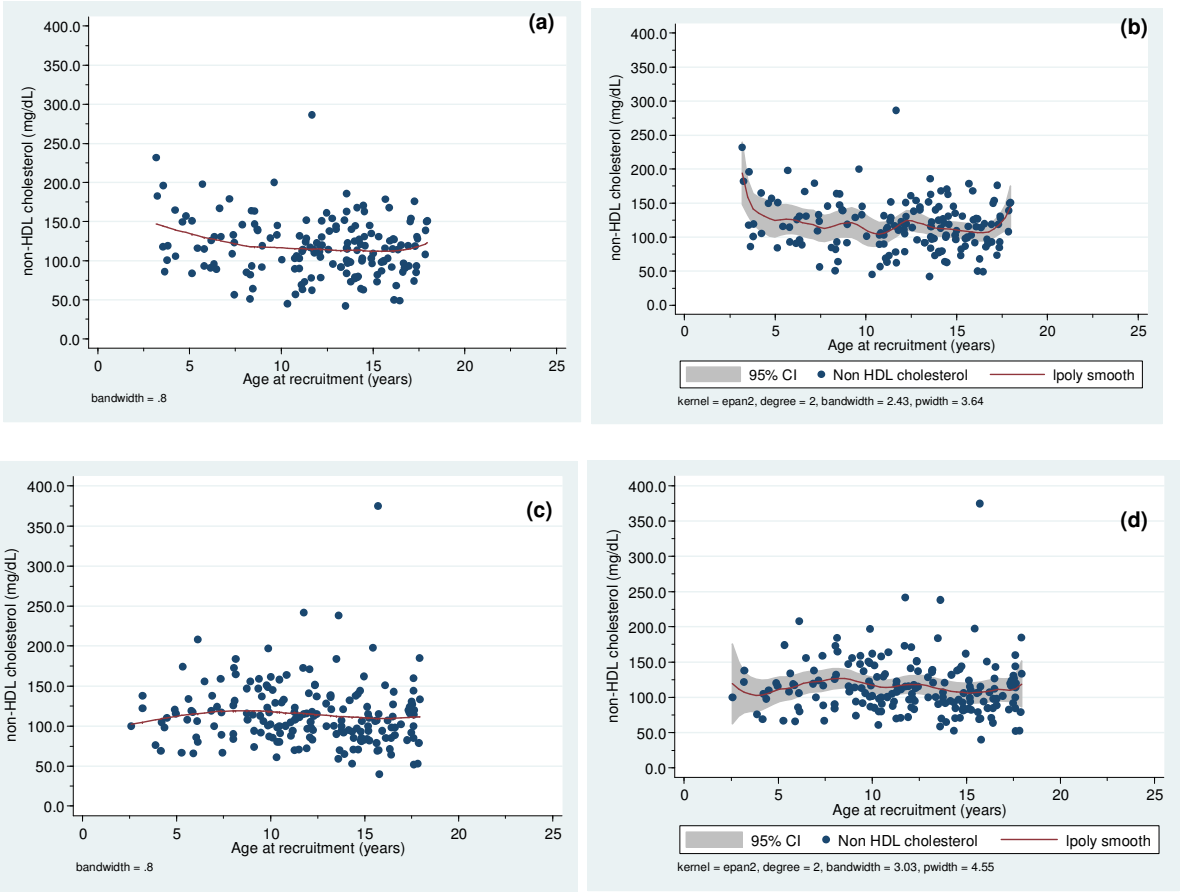
Boxes demark 25th and 75th percentiles (2nd and 3rd quartile) and lines indicate adjacent values (most extreme values within 1.5 interquartile range of the nearest quartile) of the distribution within each age band. Values outside adjacent values excluded for clarity. *n* =360 for non-HDL cholesterol, and *n* = 351 for standardized non-HDL cholesterol. Comparison of median standardized non- HDL-cholesterol between males and females: *p* > 0.05 for all groups.

Figure C-15: Comparison of median non-HDL cholesterol across age groups between subjects with/without (a) lipodystrophy syndrome (*n* = 341), (b) fat disorder (*n* = 343), and (c) metabolic abnormality (*n* = 341)



Bars indicate 25th and 75th percentiles of the distribution with each age band. No significant differences were seen in median non-HDL cholesterol between subjects with fat disorder and those without ($p > 0.05$)

Figure C-16: Modelling non high density lipoprotein (HDL) cholesterol as a function of age at recruitment: in males estimated by (a) locally weighted smoothing, (b) fractional polynomial model: and, in females estimated by (c) locally weighted smoothing, and (d) fractional polynomial model.

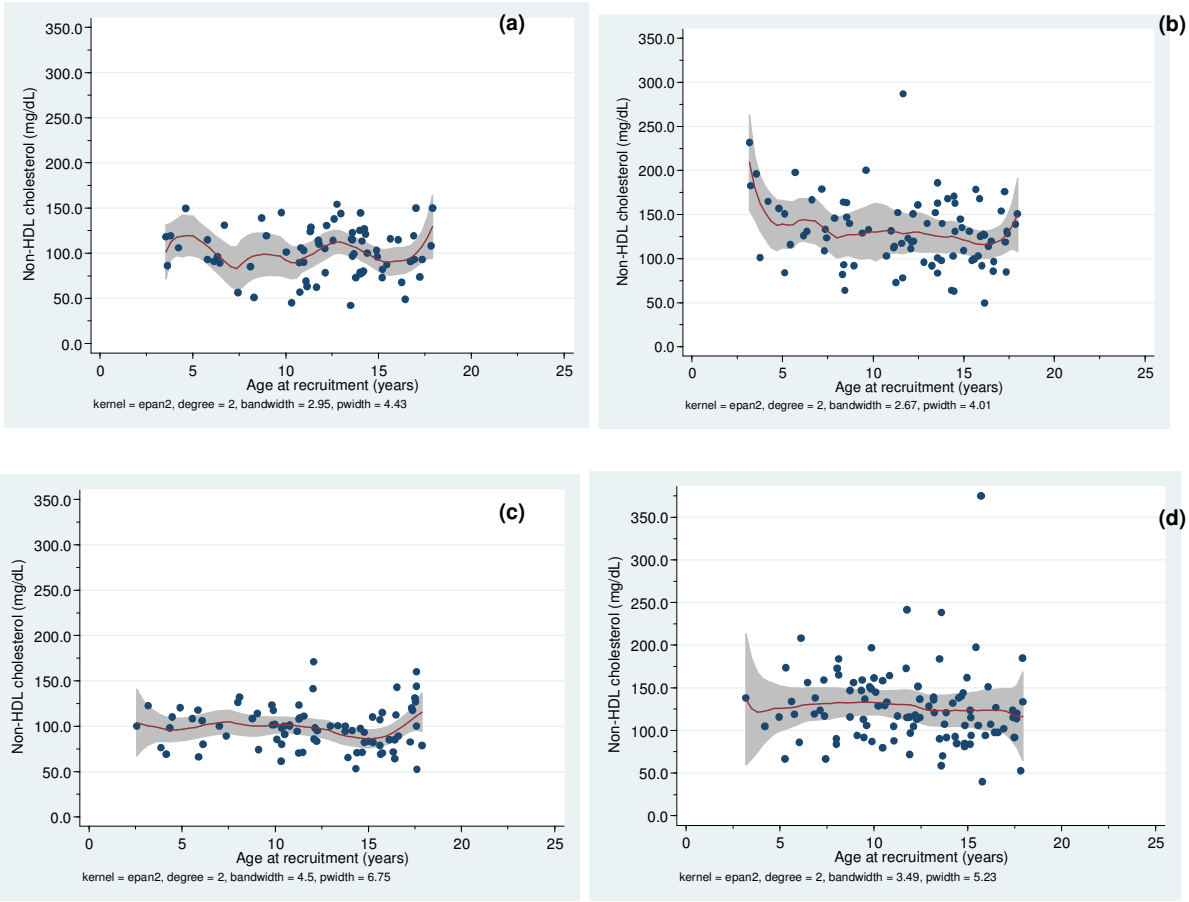


Both fractional polynomial models are order 2, estimated over 44 models. Fractional polynomial model for males: $y_i = \alpha_i + {}^{\beta_{1i}}/_{x_i^2} + \beta_{2i}x_i^3$**: (*n* = 163). Fractional polynomial model for females:** $y_i = \alpha_i + {}^{\beta_{1i}}/_{x_i^2} + \beta_{2i}x_i^3$ **: (*n* = 188).**

Table C-13: Fractional polynomial models fitting non-HDL-cholesterol as a function of age at recruitment, stratified by sex and lipodystrophy syndrome status

Outcome	Sex	Model	*n*	Coefficients			
				Parameter	Estimate	95% confidence interval	*p*-value
No lipodystrophy syndrome	**Male**	$y_i = \alpha_i + {\beta_{1i}}/{x_i^2} + \beta_{2i}x_i^3$	73	α	51.97	47.68, 56.26	<0.001
				β_1	0.75	-1.98, 3.48	0.587
				β_2	-2.16	-4.98, 0.65	0.130
	Female	$y_i = \alpha_i + \beta_{1i}x_i^3 + \beta_{2i}x_i^3(ln(x_i))$	81	α	56.69	51.61, 61.77	<0.001
				β_1	0.88	-8.91, 10.67	0.859
				β_2	-3.32	-19.20, 12.55	0.678
Lipodystrophy syndrome	**Male**	$y_i = \alpha_i + {\beta_{1i}}/{x_i^2} + \beta_{2i}x_i^3$	89	α	49.26	45.38, 53.14	<0.001
				β_1	0.65	-1.41, 2.72	0.529
				β_2	-1.81	-4.11, 0.49	0.122
	Female	$y_i = \alpha_i + {\beta_{1i}}/{x_i^2} + {\beta_{2i}(ln(x_i))}/{x_i^2}$	105	α	53.21	49.94, 56.48	<0.001
				β_1	-1.01	-10.65, 8.62	0.835
				β_2	-2.64	-11.81, 6.53	0.570

Figure C-17: Modelling non-HDL cholesterol as a function of age at recruitment in: (a) males without lipodystrophy syndrome (*n* = 72), (b) males with lipodystrophy syndrome (*n* = 88), (c) females without lipodystrophy syndrome (*n* = 80), and (d) female with lipodystrophy syndrome (*n* = 101)



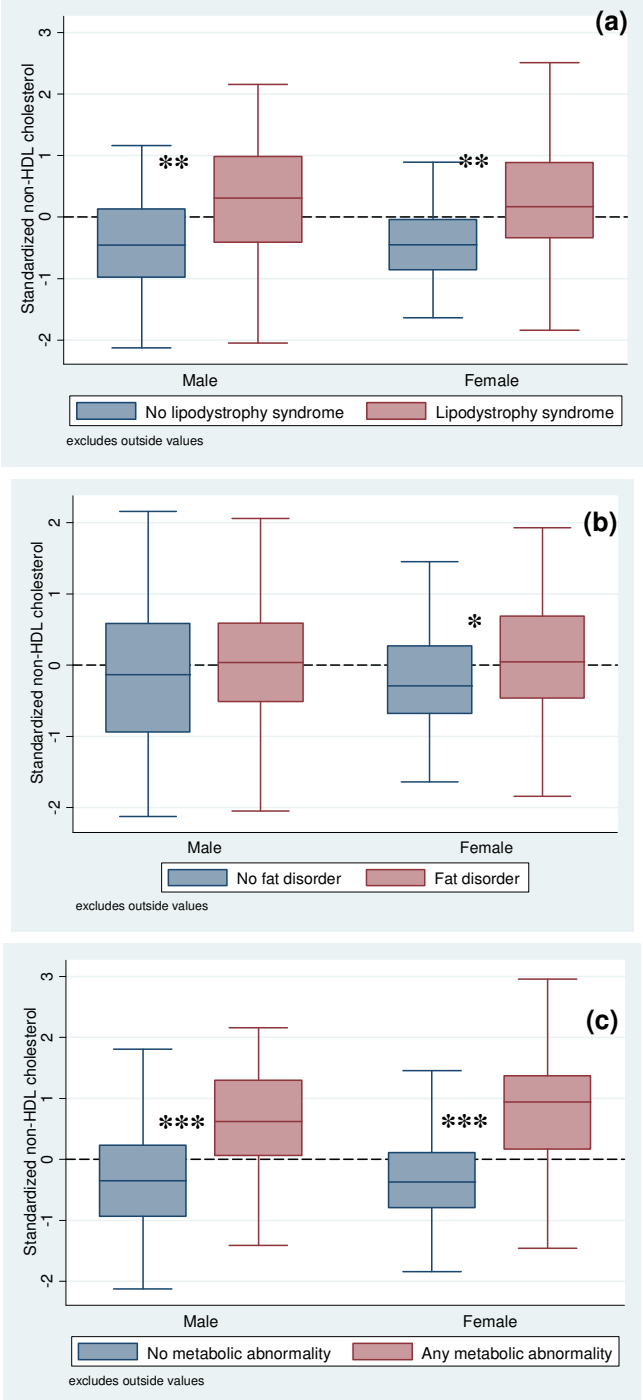
Equations: (a) $y_i = \alpha_i + {\beta_{1i}}/{x_i^2} + \beta_{2i}x_i^3$ **(b)** $y_i = \alpha_i + \beta_{1i}x_i^3 + \beta_{2i}x_i^3(ln(x_i))$ **(c)** $y_i = \alpha_i + {\beta_{1i}}/{x_i^2} + \beta_{2i}x_i^3$ **and (d)** $y_i = \alpha_i + {\beta_{1i}}/{x_i^2} + {\beta_{2i}(ln(x_i))}/{x_i^2}$

. Fractional polynomial models are of order 2, estimated over 44 models. Shaded area denotes 95% confidence interval. See Table C-13 for details of models.

Table C-14: Fractional polynomial models fitting non-HDL-cholesterol as a function of age at recruitment, stratified by sex and metabolic abnormality status

Outcome	Sex	Model	*n*	Coefficients			
				Parameter	Estimate	95% confidence interval	*p*-value
No metabolic abnormality	**Male**	$y_i = {\beta_{1i}}/{x_i^2} + \beta_{2i}x_i^3$	119	α	52.17	49.02, 55.32	<0.001
				β_1	0.78	-1.66, 3.23	0.527
				β_2	-1.84	-3.91, 0.22	0.080
	Female	$y_i = \alpha_i + \beta_{1i}x_i^3 + \beta_{2i}x_i^3 ln(x_i)$	139	α	56.84	52.92, 60.76	<0.001
				β_1	2.24	-5.39, 10.67	0.517
				β_2	-6.04	-18.87, 6.79	0.354
Metabolic abnormality	**Male**	$y_i = {\beta_{1i}}/{x_i^2} + \beta_{2i}x_i^3$	43	α	45.92	40.08, 51.76	<0.001
				β_1	0.98	-1.25, 3.21	0.380
				β_2	-3.33	-6.70, 0.04	0.052
	Female	$y_i = \alpha_i + \beta_{1i}x_i^3 + \beta_{2i}x_i^3 ln(x_i)$	47	α	49.44	44.12, 54.77	<0.001
				β_1	-16.47	-29.39, -3.54	0.014
				β_2	27.89	5.62, 50.17	0.015

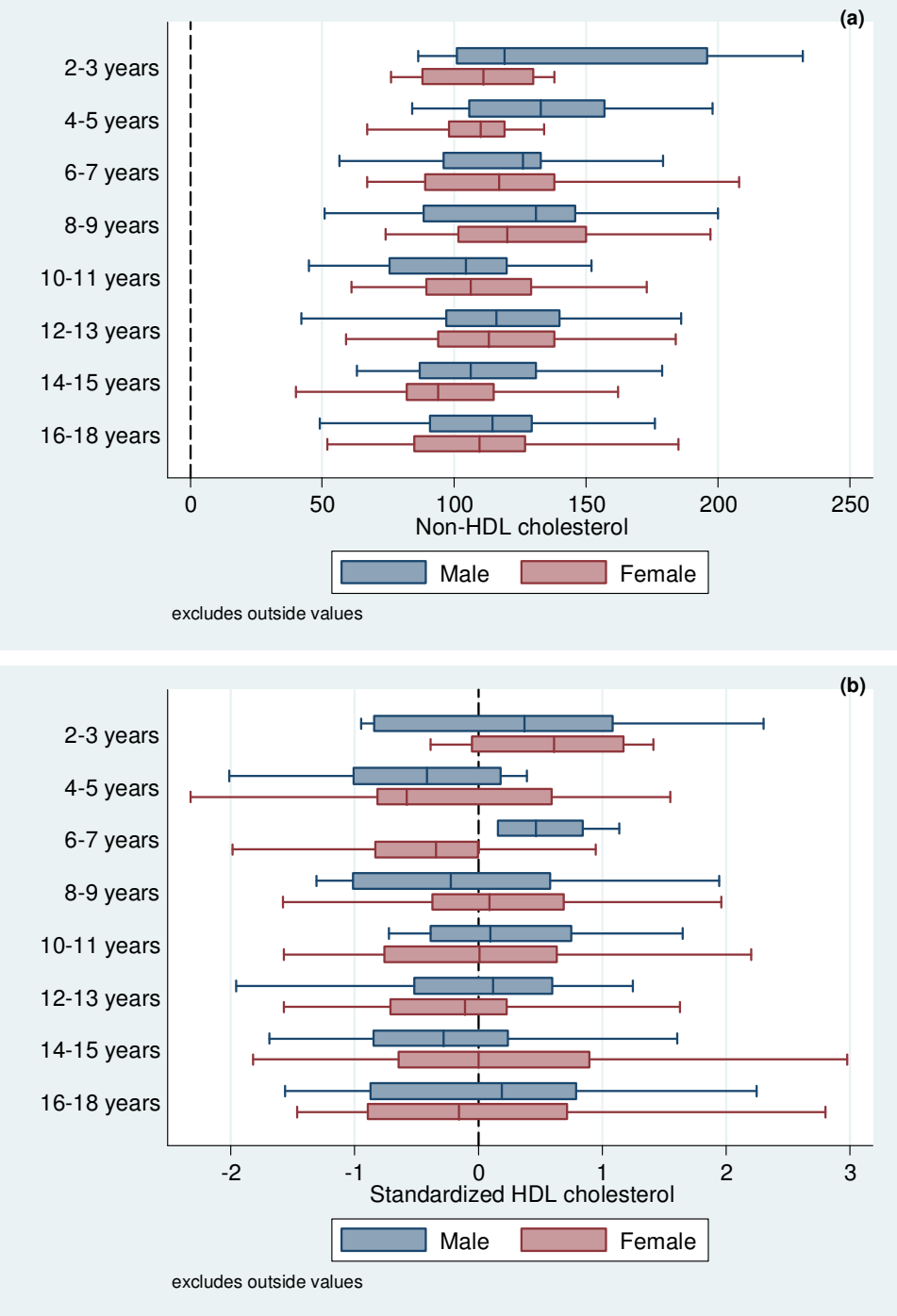
Figure C-18: Comparison of median standardized non HDL-cholesterol, stratified by sex, between subjects with/without (a) lipodystrophy syndrome, (b) fat disorder, and (c) metabolic abnormality



Boxes demark 25th and 75th percentiles (2nd and 3rd quartile) and lines indicate adjacent values (most extreme values within 1.5 interquartile range of the nearest quartile) of the distribution within each age band. Values outside adjacent values excluded for clarity. Comparison of median standardized non-HDL cholesterol ratio between subjects with outcome and without outcome: *$p < 0.01$, **$p < 0.01$, ***$p < 0.001$.

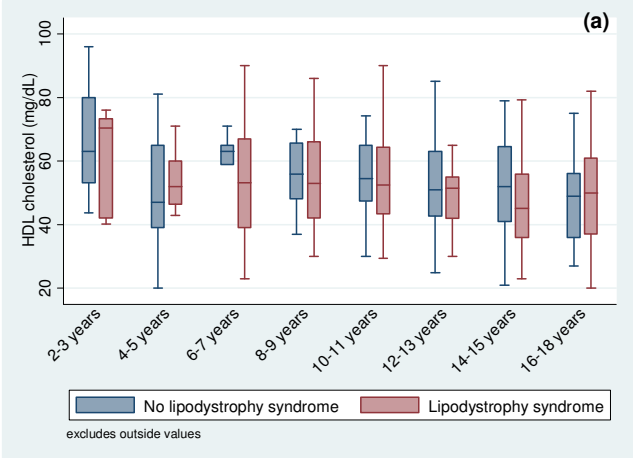
C.10 High density lipoprotein cholesterol

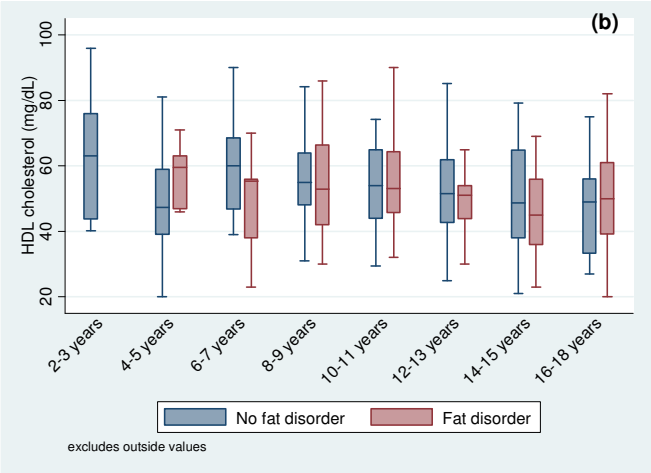
Figure C-19: High density lipoprotein (HDL) cholesterol across age groups: (a) median HDL cholesterol, and (b) median standardized HDL cholesterol

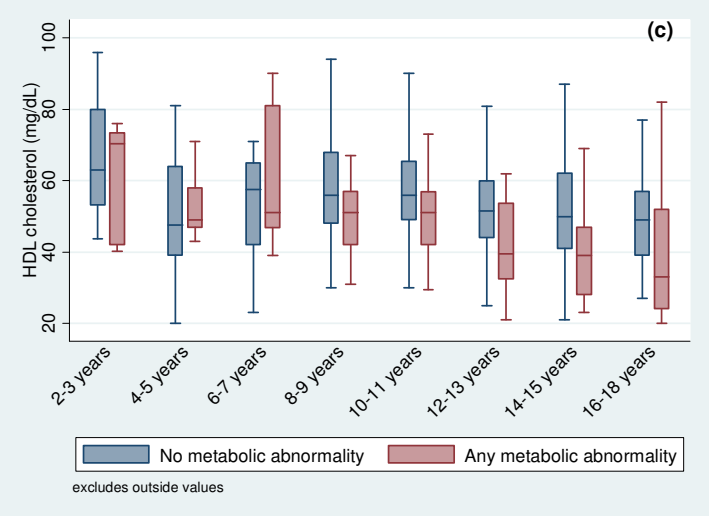


Boxes demark 25th and 75th percentiles (2nd and 3rd quartile) and lines indicate adjacent values (most extreme values within 1.5 interquartile range of the nearest quartile) of the distribution within each age band. Values outside adjacent values excluded for clarity. No significant difference in standardized HDL-cholesterol was seen between males and females in 2-3, 4-5, 8-9, 10-11, 12-13, 14-15, 16-18 year olds ($p < 0.05$): significant difference seen in 6-7 year olds ($p = 0.019$).

Figure C-20: Comparison of median high density lipoprotein (HDL) cholesterol across age groups between subjects with/without (a) lipodystrophy syndrome, (b) fat disorder, and (c) metabolic abnormality

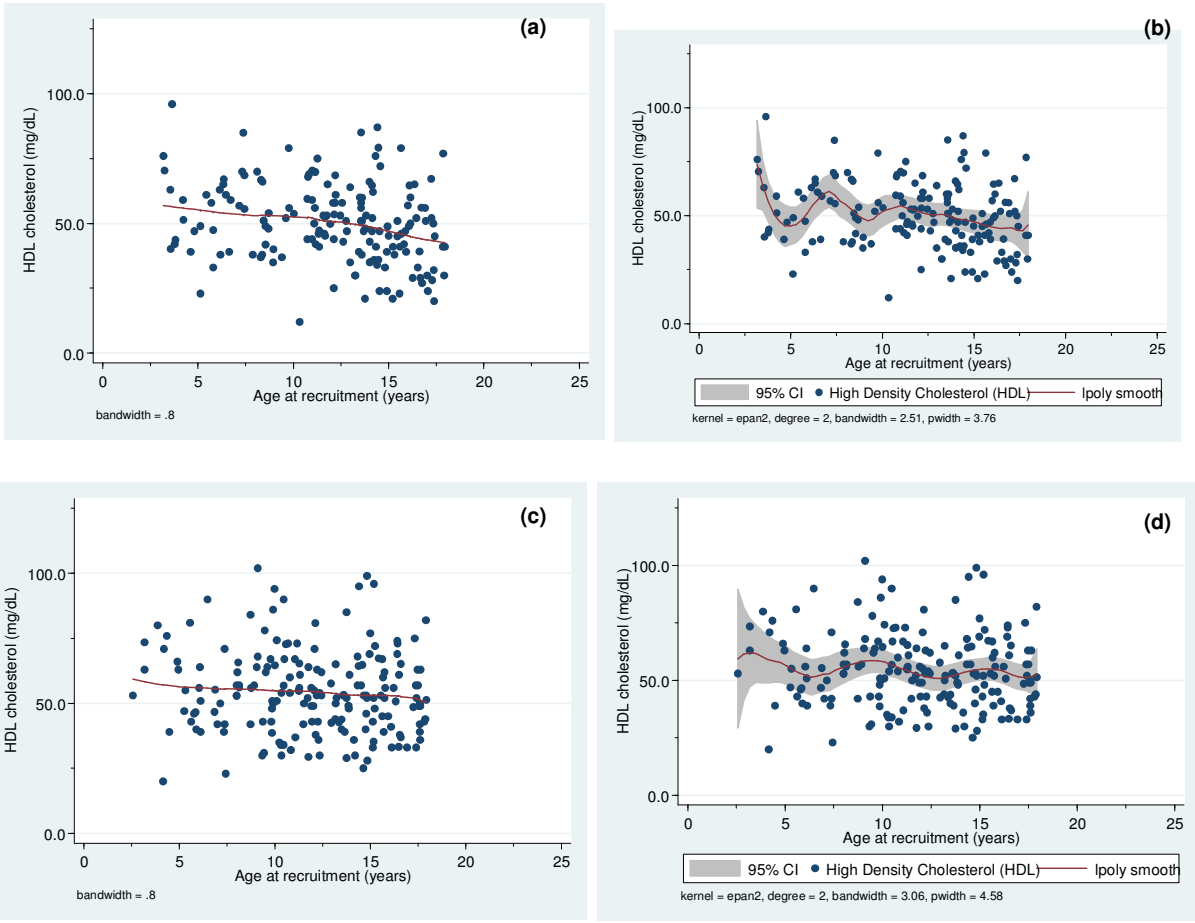






Bars indicate 25th and 75th percentiles of the distribution within each age band. No significant differences were seen in median HDL cholesterol between subjects with fat disorder and those without ($p > 0.05$)

Figure C-21: Modelling HDL-cholesterol as a function of age at recruitment in: males estimated by (a) locally weighted smoothing (b) fractional polynomial model: and, in females estimated by (c) locally weighted smoothing, and (d) fractional polynomial model

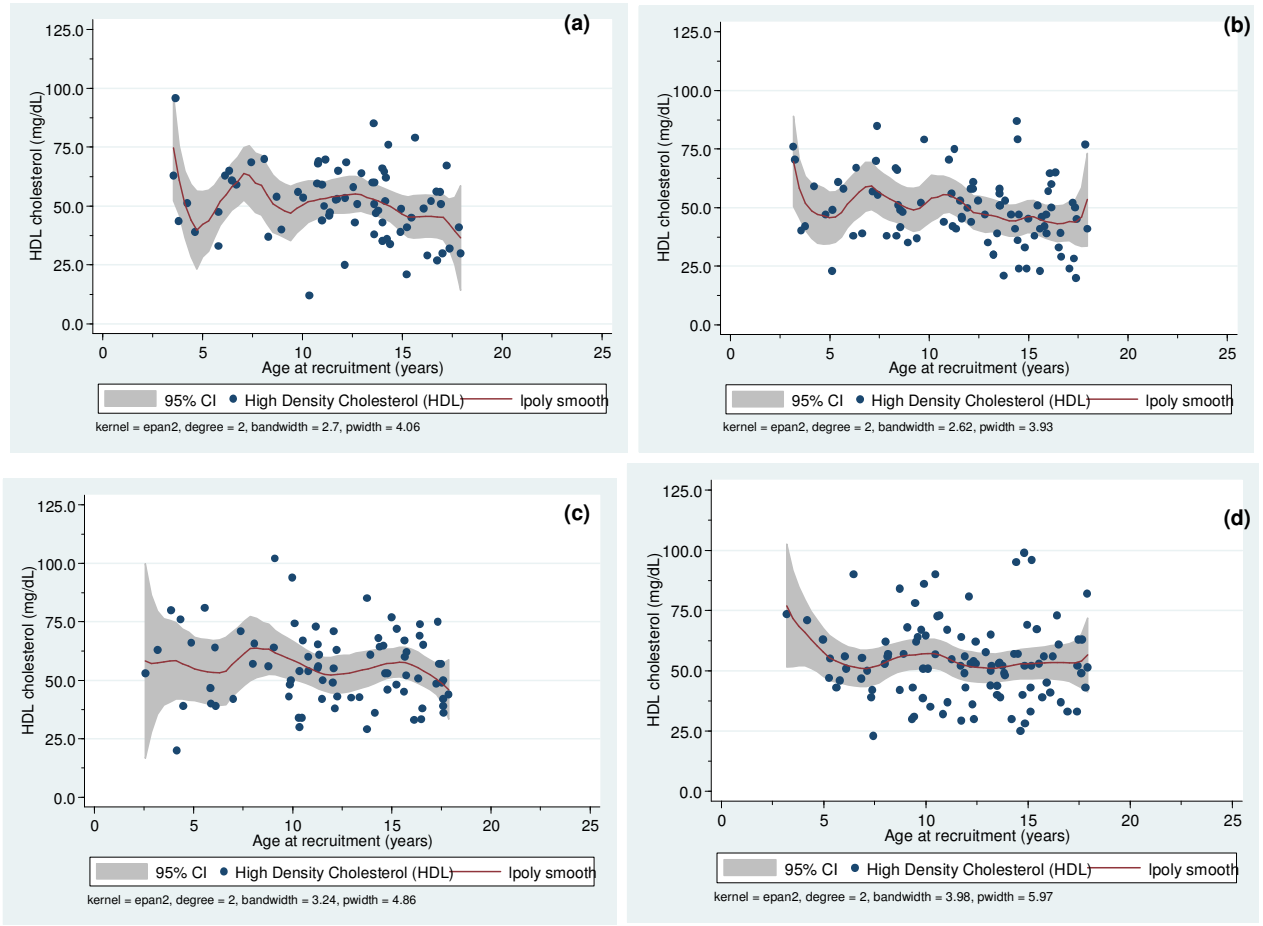


Both fractional polynomial models are order 2, estimated over 44 models. Fractional polynomial model for males: $y_i = \alpha_i + \frac{\beta_{1i}}{x_i^2} + \frac{\beta_{2i}}{x_i^2}(\ln(x_i))$**: (**$n$ **= 163). Fractional polynomial model for females:** $y_i = \alpha_i + \beta_{1i}x_i + \beta_{2i}ln(x_i)ln(x_i)$**: (**$n$ **=188).**

Table C-15: Fractional polynomial models fitting HDL-cholesterol as a function of age at recruitment, stratified by sex and lipodystrophy syndrome status

Outcome	Sex	Model	N	Coefficients			
				Parameter	Estimate	95% confidence interval	*p*-value
No lipodystrophy syndrome	**Male**	$y_i = \alpha_i + {}^{\beta_{1i}}/_{\sqrt[2]{x_i}} + \beta_{2i} ln(x_i)$	73	α	99.30	91.71, 106.88	<0.001
				β_1	108.22	-147.63, 364.06	0.402
				β_2	55.82	-85.55, 197.20	0.434
	Female	$y_i = \alpha_i + \beta_{1i} x_i^3 + \beta_{2i} x_i^3 ln(x_i)$	81	α	94.17	86.49, 101.84	<0.001
				β_1	-10.20	-24.98, 4.57	0.173
				β_2	16.87	-7.09, 40.84	0.165
Lipodystrophy syndrome	**Male**	$y_i = \alpha_i + {}^{\beta_{1i}}/_{x_i^2} + {}^{\beta_{2i}(ln(x_i))}/_{x_i^2}$	89	α	125.27	116.94, 133.59	<0.001
				β_1	1.30	-21.21, 23.81	0.909
				β_2	-5.19	-25.32, 14.94	0.610
	Female	$y_i = \alpha_i + \beta_{1i} \sqrt[2]{x_i} + \beta_{2i} x_i$	105	α	129.18	118.08, 140.27	<0.001
				β_1	155.80	-335.12, 646.73	0.530
				β_2	-84.69	-323.32, 153.95	0.483

Figure C-22: Modelling HDL cholesterol as a function of age in recruitment in: (a) males without lipodystrophy syndrome (*n* = 73), (b) males with lipodystrophy syndrome (*n*=89), (c) females without lipodystrophy syndrome (*n* = 81), (d) female with lipodystrophy syndrome (*n*=105)

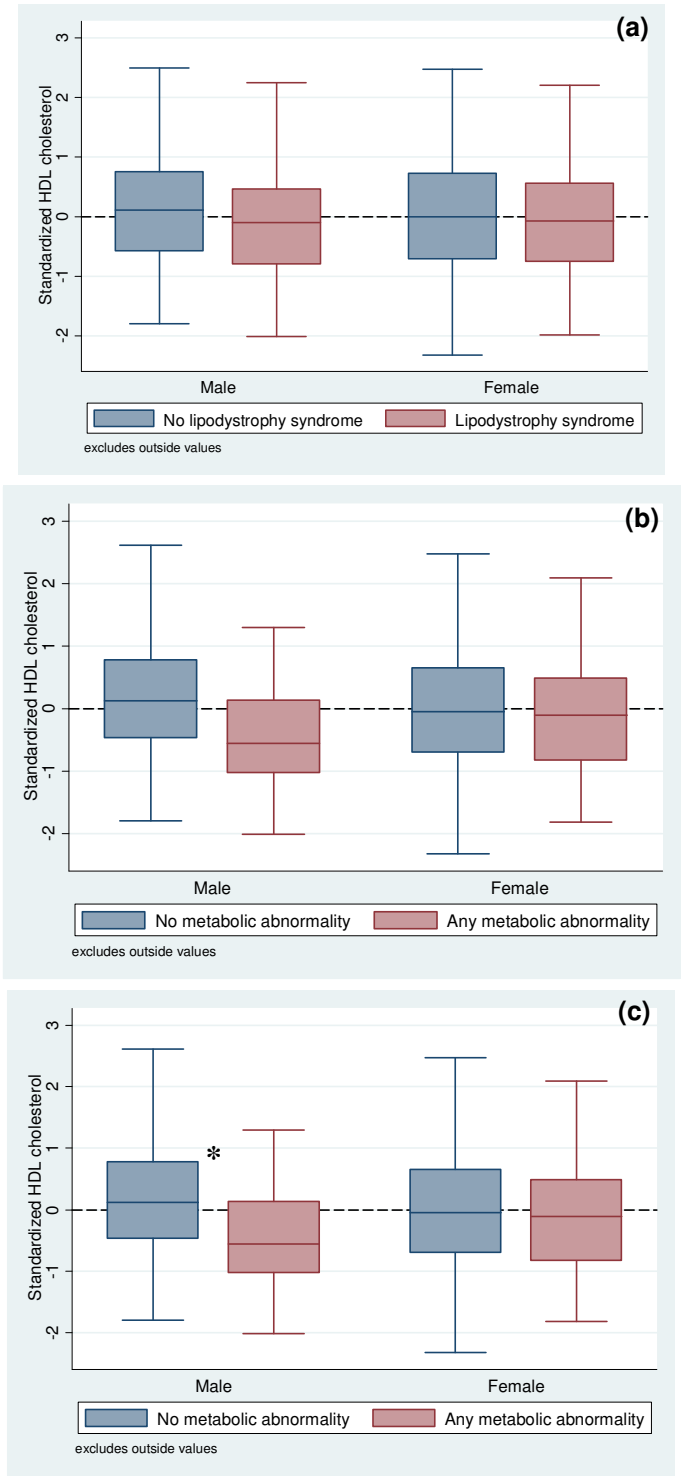


Equations: (a) $y_i = \alpha_i + {}^{\beta_{1i}}/_{x_i^2} + \beta_{2i}x_i^3$ **(b)** $y_i = \alpha_i + {}^{\beta_{1i}}/_{x_i^2} + \beta_{2i}x_i^3$ **(c)** $y_i = \alpha_i + \beta_{1i}x_i^3 + \beta_{2i}x_i^3(ln(x_i))$ **and (d)** $y_i = \alpha_i + {}^{\beta_{1i}}/_{x_i^2} + {}^{\beta_{2i}(ln(x_i))}/_{x_i^2}$ **Fractional polynomial models are of order 2, estimated over 44 models. Shaded area denotes 95% confidence interval. See Table C-15 for details of models.**

Table C-16: Fractional polynomial models fitting HDL-cholesterol as a function of age at recruitment, stratified by sex and metabolic abnormality status

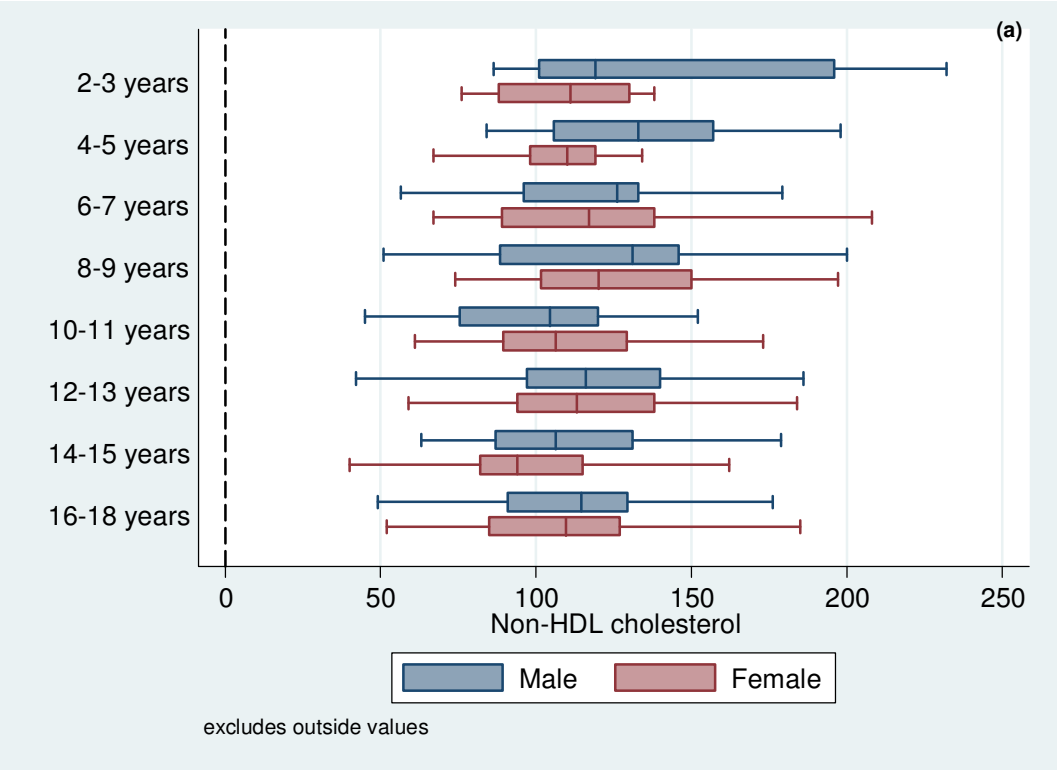
Outcome	Sex	Model	*n*	Coefficients			
				Parameter	Estimate	95% confidence interval	*p*-value
No metabolic abnormality	**Male**	$y_i = \alpha_i + \beta_{1i}\sqrt[2]{x_i} + \beta_{2i}x_i^3$	119	α	103.83	96.79, 110.87	<0.001
				β_1	-30.41	-101.53, 40.72	0.399
				β_2	3.55	-4.55, 11.66	0.387
	Female	$y_i = \alpha_i + \beta_{1i}x_i^3 + \beta_{2i}x_i^3(ln(x_i))$	139	α	100.86	92.61, 109.11	<0.001
				β_1	-2.39	-19.29, 14.50	0.780
				β_2	5.39	-21.60, 32.39	0.693
Metabolic abnormality	**Male**	$y_i = \alpha_i + \beta_{1i}/x_i^2 + \beta_{2i}(ln(x_i))/x_i^2$	43	α	141.50	127.57, 155.43	<0.001
				β_1	-7.31	-40.92, 26.31	0.663
				β_2	-10.64	-39.74, 18.46	0.464
	Female	$y_i = \alpha_i + \beta_{1i}\sqrt[2]{x_i} + \beta_{2i}\sqrt[2]{x_i}\,(ln(x_i))$	47	α	151.78	138.86, 164.70	<0.001
				β_1	226.89	-267.68, 721.46	0.360
				β_2	-106.78	-361.07, 148.71	0.360

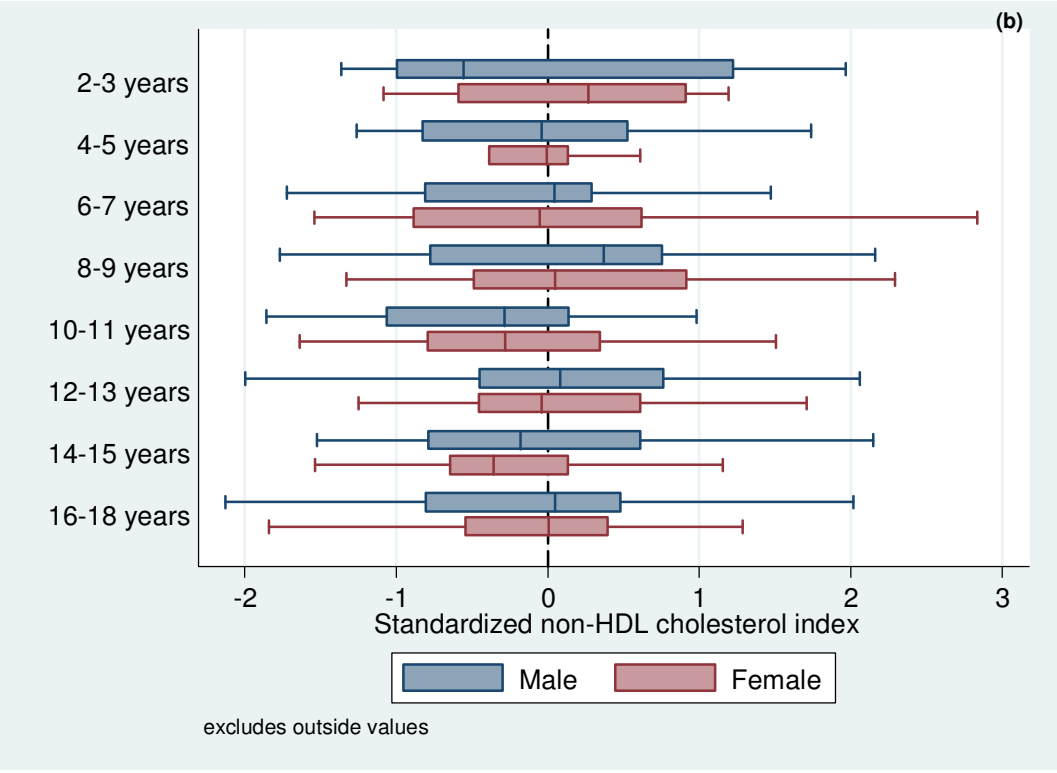
Figure C-23: Comparison of median standardized HDL-cholesterol, stratified by sex, between subjects with/without (a) lipodystrophy syndrome, (b) fat disorder, and (c) metabolic abnormality



Boxes demark 25th and 75th percentiles (1st and 3rd quartile) and lines indicate adjacent values (most extreme values within 1.5 interquartile range of the nearest quartile) of the distribution within each age band. Values outside adjacent values excluded for clarity. Comparison of median standardized HDL-cholesterol ratio between subjects with outcome and without outcome: *$p < 0.01$, **$p < 0.01$, ***$p < 0.001$.

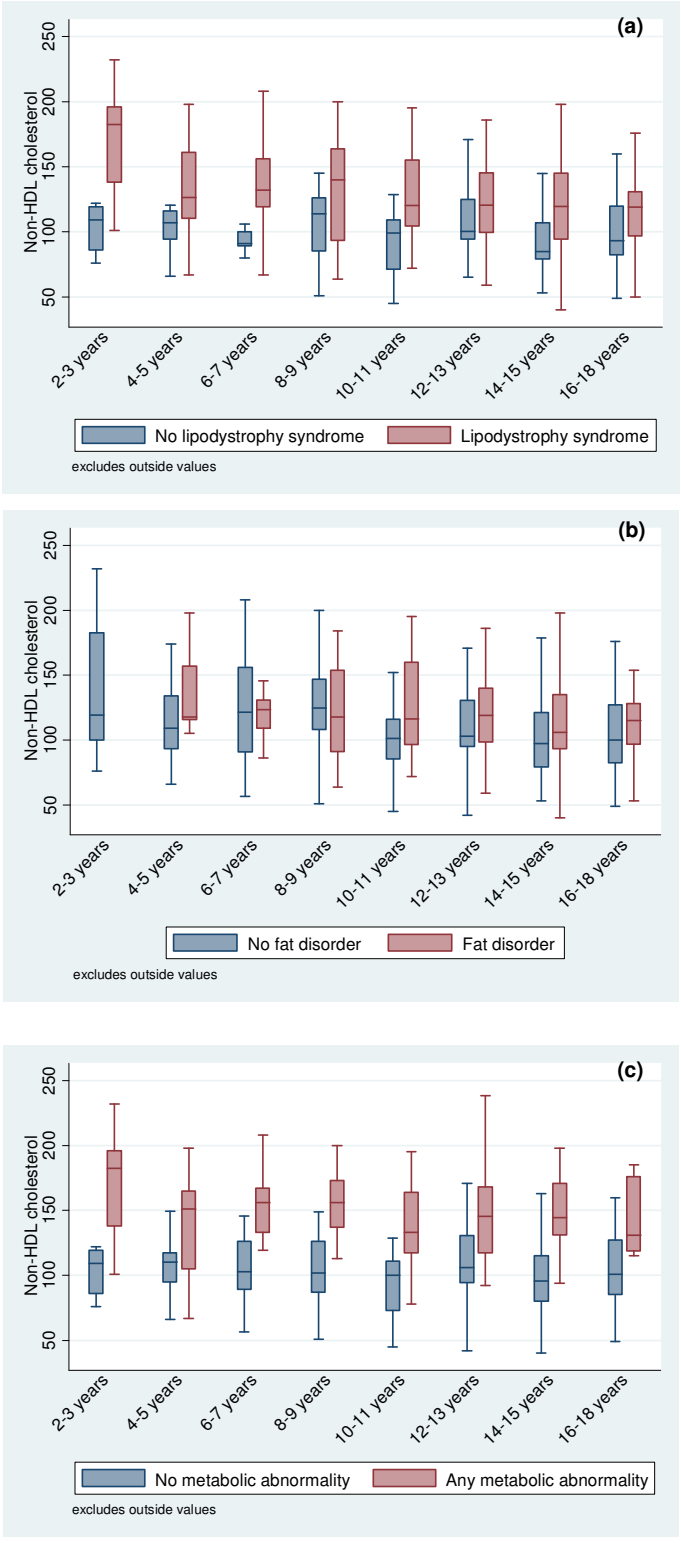
Figure C-24: Non high density lipoprotein cholesterol across age groups: (a) median non-HDL cholesterol, and (b) median standardized non-HDL cholesterol





Boxes demark 25th and 75th percentiles (2nd and 3rd quartile) and lines indicate adjacent values (most extreme values within 1.5 interquartile range of the nearest quartile) of the distribution within each age band. Values outside adjacent values excluded for clarity.. *n* =360 for non-HDL cholesterol, and *n* = 351 for standardized non-HDL cholesterol. Comparison of median standardized non- HDL-cholesterol between males and females: *p* > 0.05 for all groups.

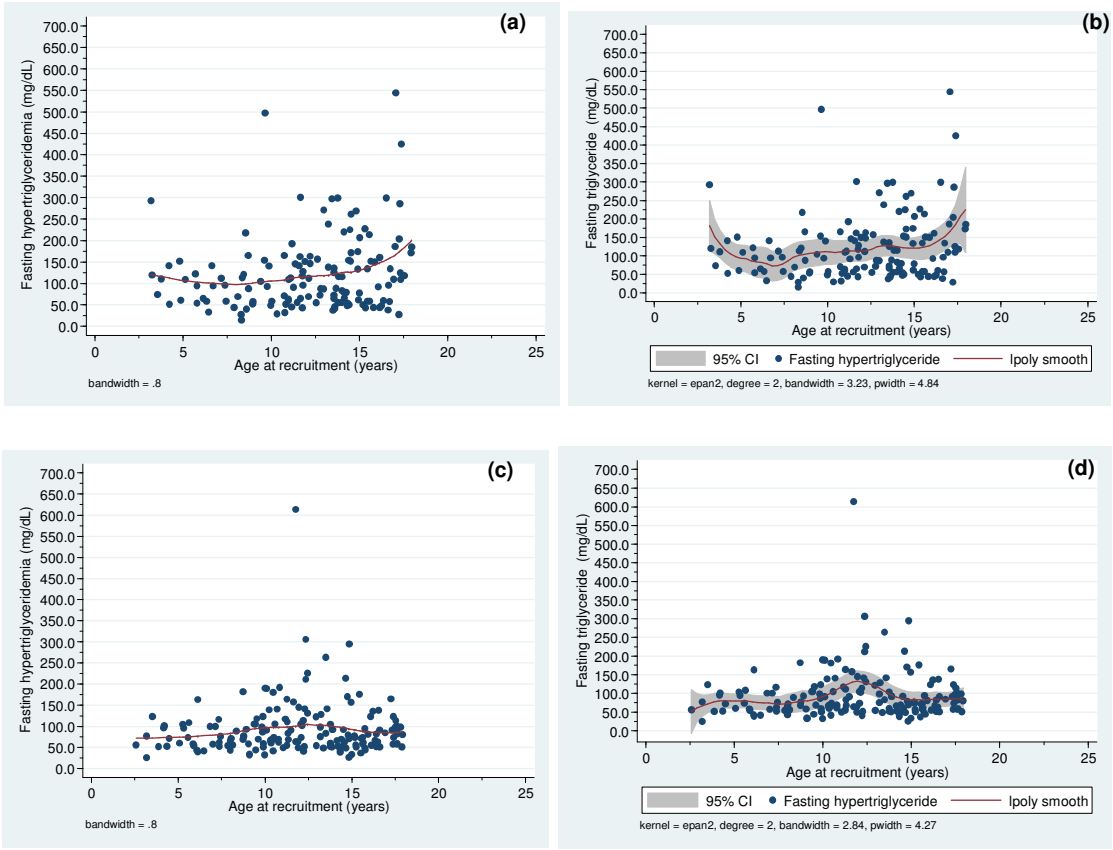
Figure C-25: Comparison of median non-HDL cholesterol across age groups between subjects with/without (a) lipodystrophy syndrome (*n* = 357), (b) fat disorder (*n* = 359), and (c) metabolic abnormality (*n* = 357)



Bars indicate 25th and 75th percentiles of the distribution with each age band. No significant differences were seen in median non-HDL cholesterol between subjects with fat disorder and those without ($p > 0.05$)

C.11 Fasting triglyceride

Figure C-26: Modelling fasting triglyceride as a function of age at recruitment in: males estimated by (a) locally weighted smoothing, (b) fractional polynomial model: and in females estimated by (c) locally weighted smoothing, and (d) fractional polynomial model.

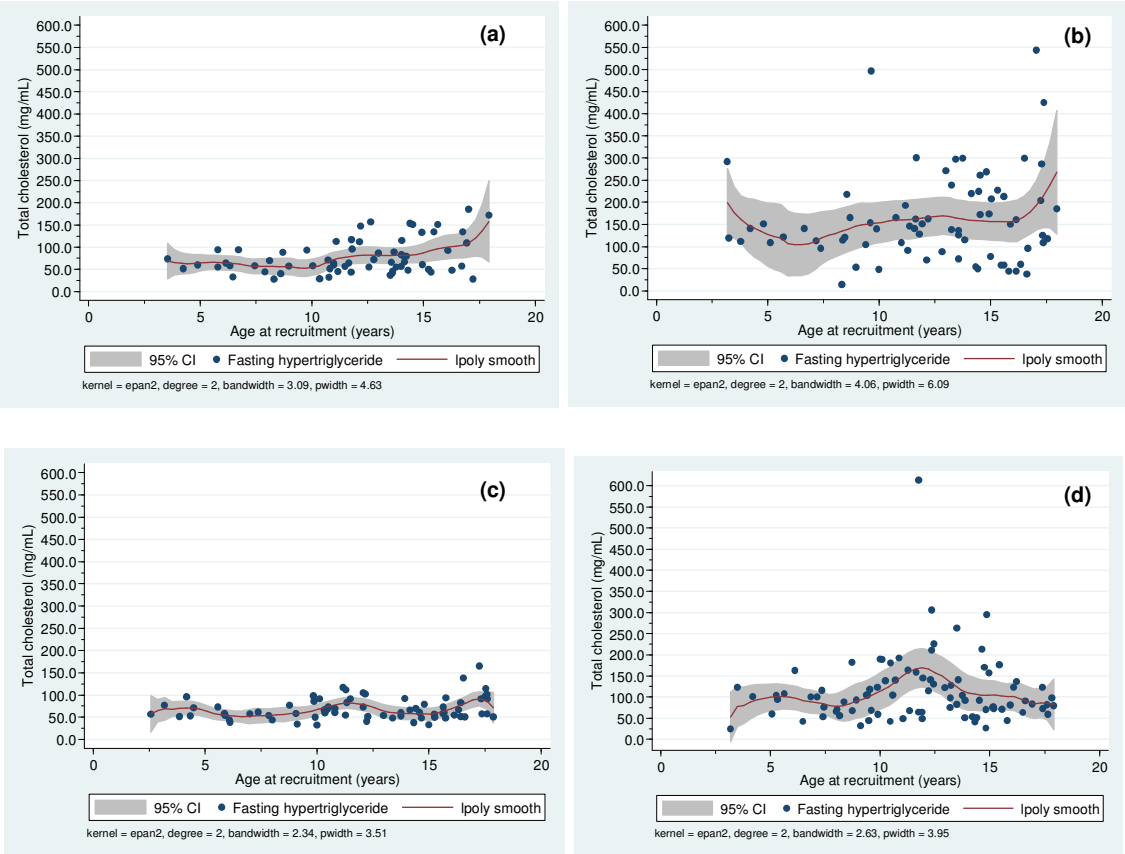


Both fractional polynomial models are order 2, estimated over 44 models. Fractional polynomial for males: $y_i = \alpha_i + {}^{\beta_{1i}}/_{x_i^2} + \beta_{2i}x_i$: ($n$ = 139). Fractional polynomial model for females: $y_i = \alpha_i + \beta_{1i}x_i^2 + \beta_{2i}x_i^3$:: ($n$ = 166). Shaded area in (b) and (d) denote 95% confidence interval.

Table C-17: Fractional polynomial models fitting fasting triglyceride as a function of age at recruitment, stratified by sex and lipodystrophy syndrome status

Outcome	Sex	Model	*n*	Coefficients			
				Parameter	Estimate	95% confidence interval	*p*-value
No lipodystrophy	**Male**	$y_i = \alpha_i + {}^{\beta_{1i}}/_{x_i} + \beta_{2i}x_i^3$	66	α	71.81	60.49, 83.14	<0.001
				β_1	8.88	-22.13, 39.89	0.569
				β_2	12.58	3.28, 21.9	0.009
	Female	$y_i = \alpha_i + \beta_i x_i^3 + \beta_{2i}x_i^3(ln(x_1))$	74	α	65.35	57.04, 73.65	<0.001
				β_1	-1.46	-17.40, 14.48	0.856
				β_2	8.11	-17.85, 34.07	0.535
Lipodystrophy	**Male**	$y_i = \alpha_i + {}^{\beta_{1i}}/_{x_i^2} + {}^{\beta_{2i}ln(x_i)}/_{x_i^2}$	73	α	162.07	137.77, 186.37	<0.001
				β_1	-62.03	-134.80, 10.74	0.094
				β_2	-54.23	-118.27, 9.84	0.096
	Female	$y_i = \alpha_i + \beta_i x_i^3 + \beta_{2i}x_i^3(ln(x_1))$	91	α	130.48	108.80, 152.16	<0.001
				β_1	56.54	8.04, 105.04	0.023
				β_2	-98.23	-178.19, -18.27	0.017

Figure C-27: Relationship between age and fasting triglyceride in: (a) males without lipodystrophy syndrome (*n* = 66), (b) males with lipodystrophy syndrome (*n* =73), (c) females without lipodystrophy syndrome (*n* = 74), and (d) female with lipodystrophy syndrome (*n* =91)



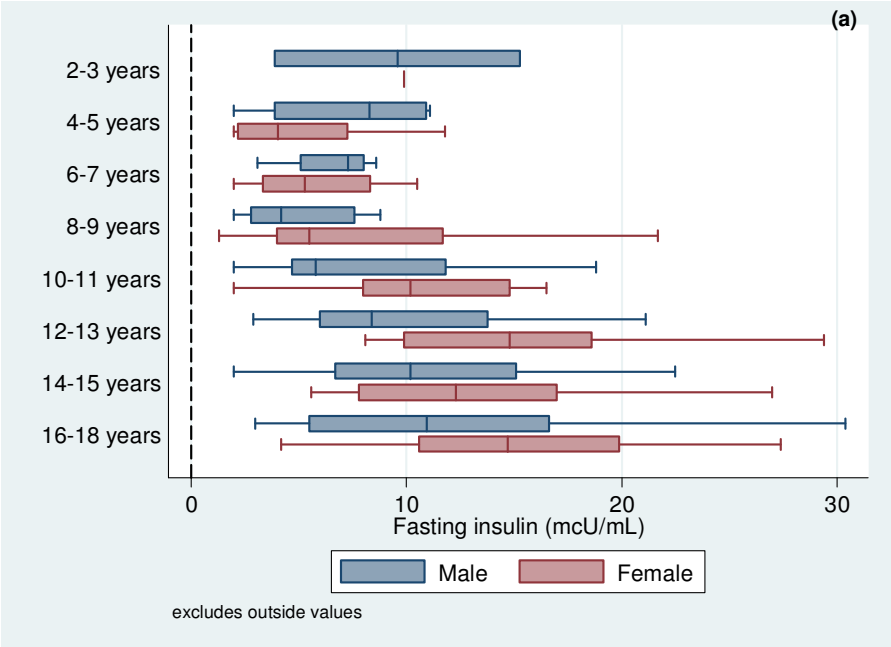
Equations: **(a)** $y_i = \alpha_i + {}^{\beta_{1i}}/_{x_i} + \beta_{2i}x_i^3$ **(b)** $y_i = \alpha_i + {}^{\beta_{1i}}/_{x_i^2} + {}^{\beta_{2i}ln(x_i)}/_{x_i^2}$ **(c)** $y_i = \alpha_i + \beta_i x_i^3 + \beta_{2i}x_i^3(ln(x_1))$ **and (d)** $y_i = \alpha_i + \beta_i x_i^3 + \beta_{2i}x_i^3(ln(x_1))$. **Fractional polynomial models includes age raised to two powers, estimated over 44 models. Shaded area denotes 95% confidence interval. S*ee* Table C-17 for details of models.**

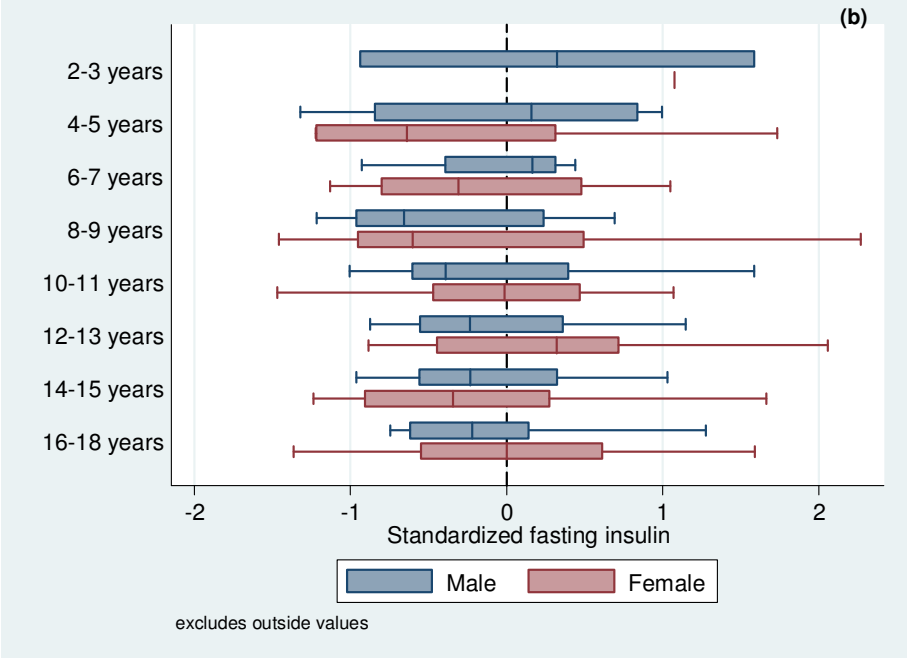
Table C-18: Fractional polynomial models fitting fasting triglyceride as a function of age at recruitment, stratified by sex and metabolic abnormality status

Outcome	Sex	Model	*n*	Coefficients			
				Parameter	Estimate	95% confidence interval	*p*-value
No metabolic abnormality	**Male**	$y_i = \alpha_i + {}^{\beta_{1i}}/_{x_i} + \beta_{2i}x_i$	94	α	80.86	71.45, 90.27	<0.001
				β_1	30.27	-19.27, 79.81	0.228
				β_2	76.23	19.79, 132.67	0.009
	Female	$y_i = \alpha_i + \beta_{1i}x_i^3 + \beta_{2i}x_i^3(ln(x_i))$	119	α	68.65	62.04, 75.27	<0.001
				β_1	2.45	-11.36, 16.26	0.726
				β_2	2.88	-19.33, 25.09	0.797
Metabolic abnormality	**Male**	$y_i = \alpha_i + {}^{\beta_{1i}}/_{x_i} + \beta_{2i}x_i$	45	α	185.20	154.44, 215.96	<0.001
				β_1	19.32	0.39, 38.26	0.046
				β_2	204	101.70, 307.11	<0.001
	Female	$y_i = \alpha_i + \beta_{1i}x_i^3 + \beta_{2i}x_i^3(ln(x_i))$	45	α	174.15	67.72, 206.57	<0.001
				β_1	137.15	67.75, 206.57	<0.001
				β_2	-224.20	-344.59, -103.82	0.001

C.12 Fasting insulin

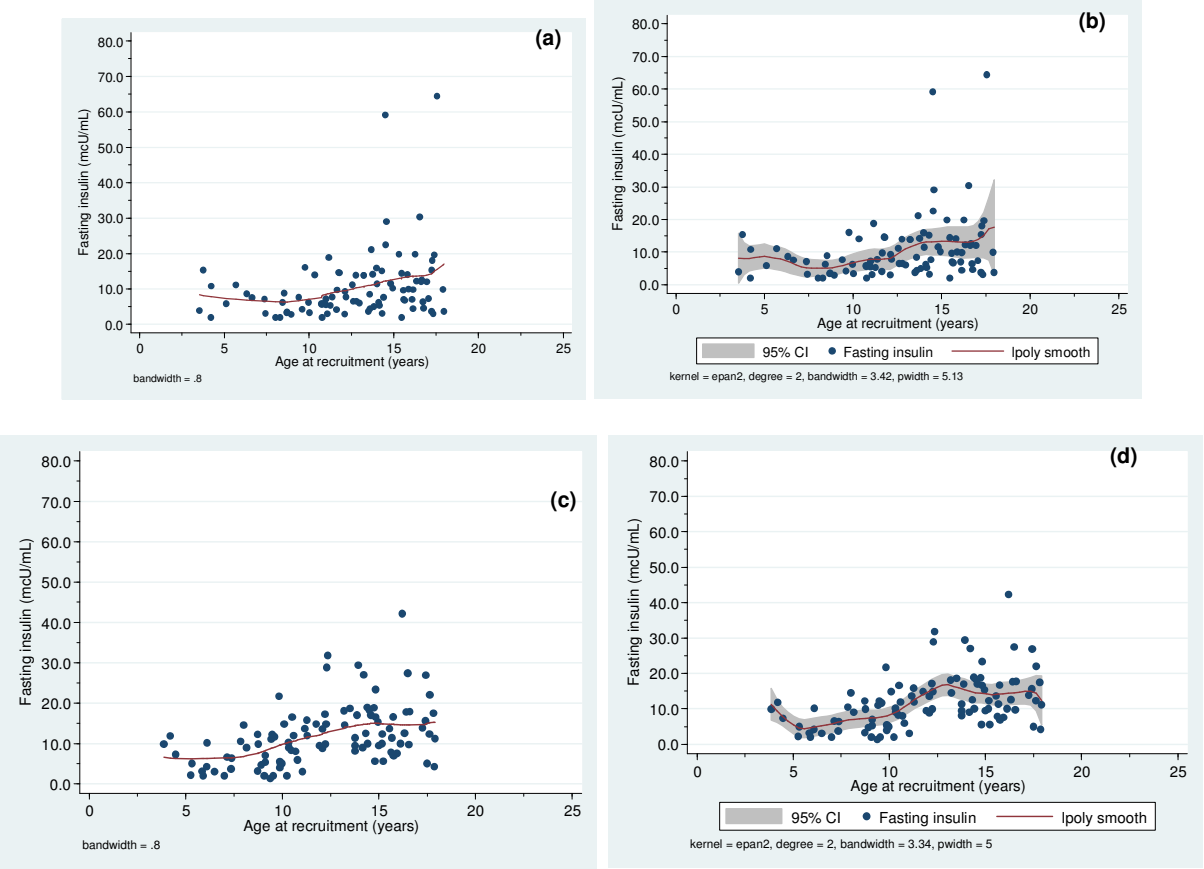
Figure C-28: Fasting insulin across age groups: (a) median fasting insulin, and (b) median standardized fasting insulin





Boxes demark 25th and 75th percentiles (2nd and 3rd quartile) and lines indicate adjacent values (most extreme values within 1.5 interquartile range of the nearest quartile) of the distribution within each age band. Values outside adjacent values excluded for clarity. *n* =199 for fasting insulin, and *n* = 196 for standardized fasting insulin respectively. Comparison of median standardized fasting insulin between males and females: $p > 0.05$ for all groups

Figure C-29: Modelling fasting insulin as a function of age at recruitment estimated: in males by (a) locally weighted smoothing, (b) fractional polynomial model: and in females by (c) locally weighted smoothing and (d) fractional polynomial model

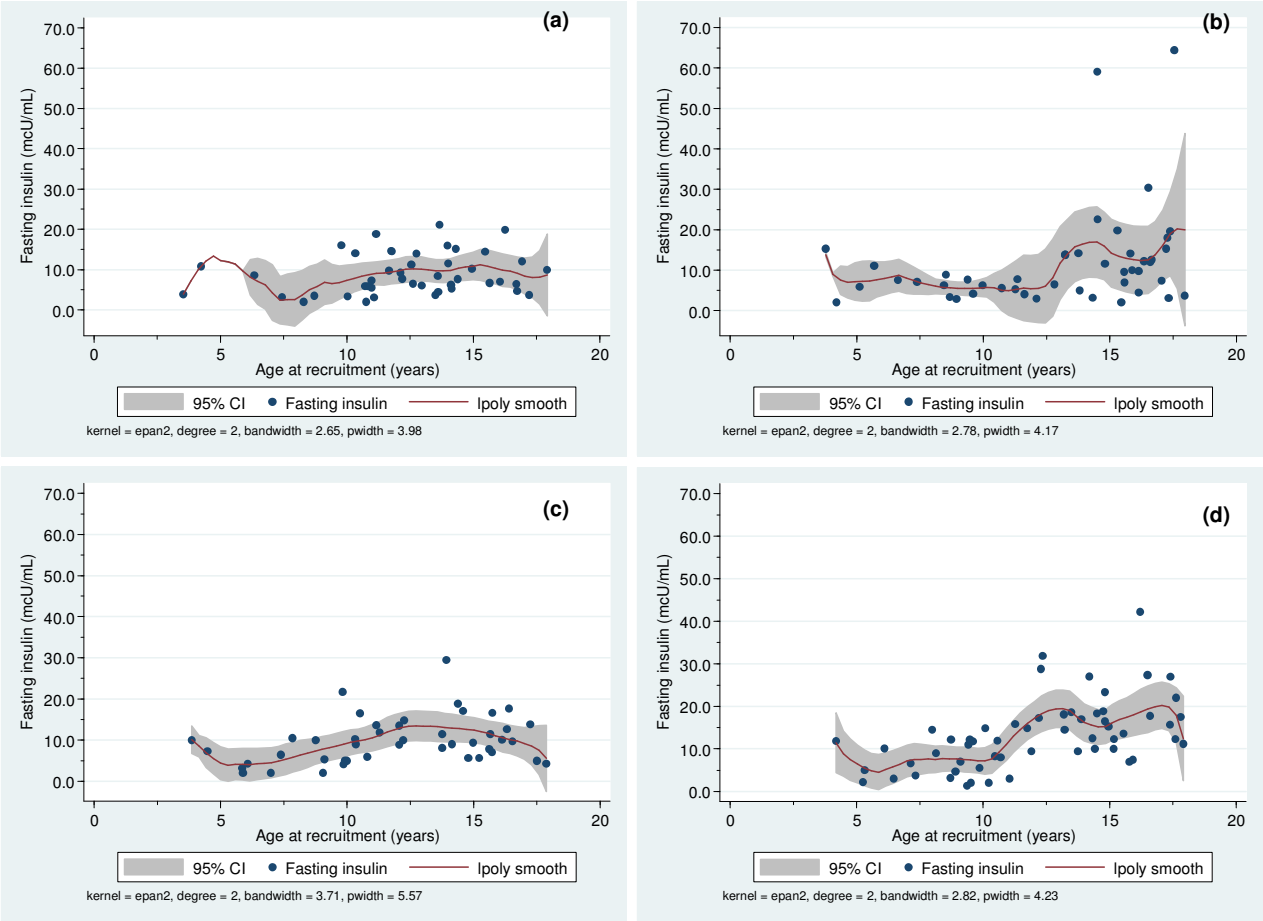


Both fractional polynomial models are order 2, estimated over 44 models. Fractional polynomial for males: $y_i = \alpha_i + \beta_{1i}x_i + \beta_{2i}x_i(ln(x_i))$: ($n$ = 93). Fractional polynomial model for females: $y_i = \alpha_i + {\beta_{1i}}/{x_i^2} + {\beta_{2i}(\ln(x_i))}/{x_i^2}$: ($n$ = 103). Shaded area in (b) and (d) denote 95% confidence interval.

Table C-19: Fractional polynomial models fitting fasting insulin as a function of age at recruitment, stratified by sex and lipodystrophy syndrome status

Outcome	Sex	Model	*n*	Coefficients			
				Parameter	Estimate	95% confidence interval	*p*-value
No lipodystrophy	**Male**	$y_i = \alpha_i + \beta_i x_i^3 + \beta_{2i} x_i^3 (ln(x_1))$	45	α	9.47	7.54, 8.84	<0.001
				β_1	3.78	-1.28, 8.84	0.191
				β_2	-5.31	-13.38, 2.76	0.191
	Female	$y_i = \alpha_i + \beta_i x_i^3 + \beta_{2i} x_i^3 (ln(x_1))$	45	α	11.69	9.41, 13.97	<0.001
				β_1	7.65	2.67, 12.64	0.003
				β_2	-11.55	-19.80, -3.29	0.007
Lipodystrophy	**Male**	$y_i = \alpha_i + \beta_{1i} \sqrt[2]{x}_i + \beta_{2i} \sqrt[2]{x_i} (ln(x_i)$	46	α	9.97	5.41, 14.53	<0.001
				β_1	-108.77	-289.48, 71.94	0.231
				β_2	62.98	-26.11, 152.08	0.161
	Female	$y_i = \alpha_i + \beta_{1i} / x_i^2 + \beta_{2i} ln(x_i) / x_i^2$	58	α	13.86	11.94, 15.76	<0.001
				β_1	-17.21	-24.82, -9.59	<0.001
				β_2	-16.71	-25, 60, -7.83	<0.001

Figure C-30: Modelling fasting insulin as a function of age at recruitment in: (a) males without lipodystrophy syndrome, (*n* = 45) (b) males with lipodystrophy syndrome (*n* = 46), (c) females without lipodystrophy syndrome (*n* =45), and (d) female with lipodystrophy syndrome (*n* = 58)



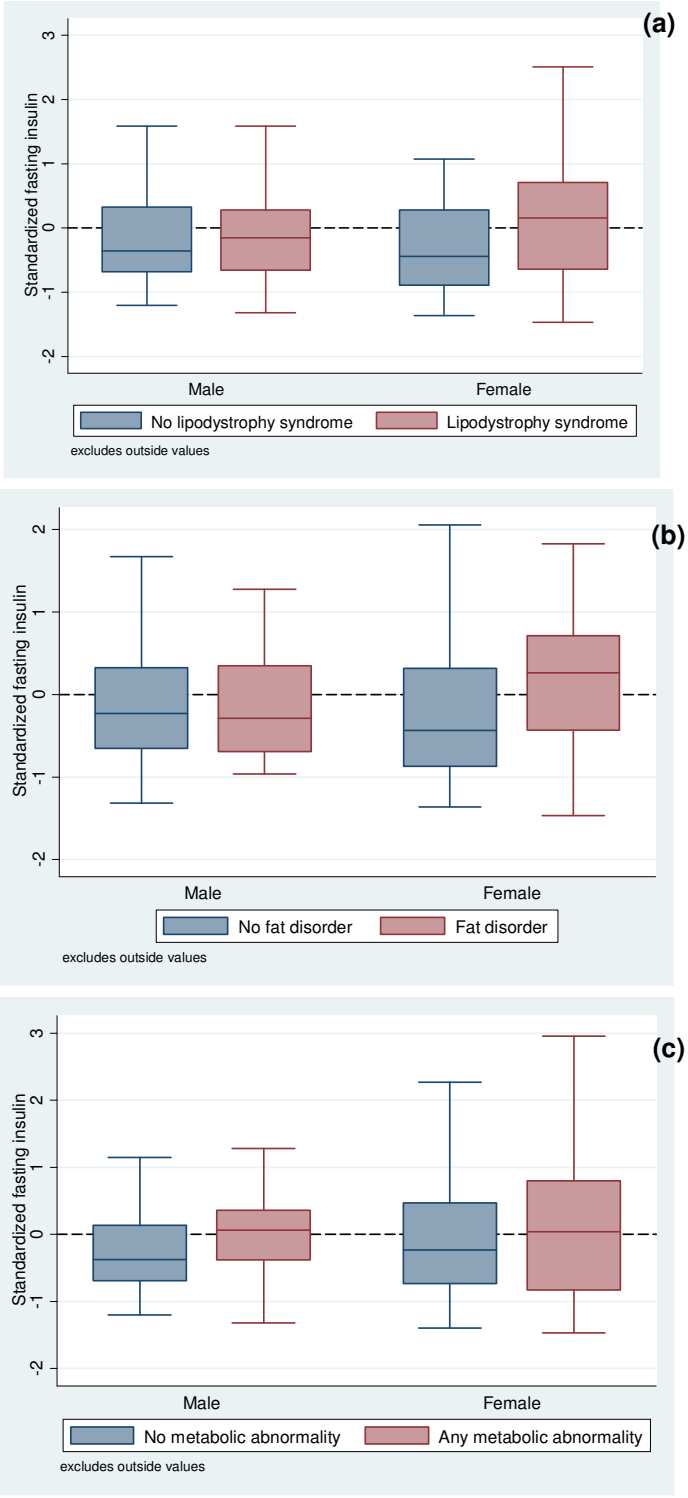
Equations: (a) $y_i = \alpha_i + \beta_i x_i^3 + \beta_{2i} x_i^3 (ln(x_1))$ **(b)** $y_i = \alpha_i + \beta_{1i} \sqrt[2]{x_i} + \beta_{2i} \sqrt[2]{x_i} (ln(x_i)$ **(c)** $y_i = \alpha_i + \beta_i x_i^3 + \beta_{2i} x_i^3 (ln(x_1))$ **and (d)** $y_i = \alpha_i + {\beta_{1i}}/{x_i^2} + {\beta_{2i} ln(x_i)}/{x_i^2}$ **Fractional polynomial models are of 2 orders, estimated over 44 models. Shaded area denotes 95% confidence interval. See Table C-19 for details of model.**

Table C-20: Fractional polynomial models fitting fasting insulin as a function of age at recruitment, stratified by sex and body metabolic abnormality status

Outcome	Sex	Model	*n*	Coefficients			
				Parameter	Estimate	95% confidence interval	*p*-value
No metabolic abnormality	**Male**	$y_i = \alpha_i + \beta_{1i}\sqrt[2]{x_i} + \beta_{2i}x_i$	64	α	9.71	6.73, 12.69	<0.001
				β_1	-46.68	-194.93, 101.57	0.531
				β_2	29.84	-41.39, 101.08	0.405
	Female	$y_i = \alpha_i + {\beta_{1i}}/{x_i^2} = {\beta_{2i}(ln(x_i))}/{x_i^2}$	75	α	12.65	11.11, 14.19	<0.001
				β_1	-11.00	-17.09, -4.91	0.001
				β_2	-9.88	-16.44, -3.31	0.004
Metabolic abnormality	**Male**	$y_i = {\beta_{1i}}/{\sqrt[2]{x_i}} + {\beta_{2i}\,(ln(x_i))}/{\sqrt[2]{x_i}}$	27	α	9.50	6.66, 12.34	<0.001
				β_1	-126.80	-219.64, -33.96	0.010
				β_2	-52.57	-94.38, -10.77	0.016

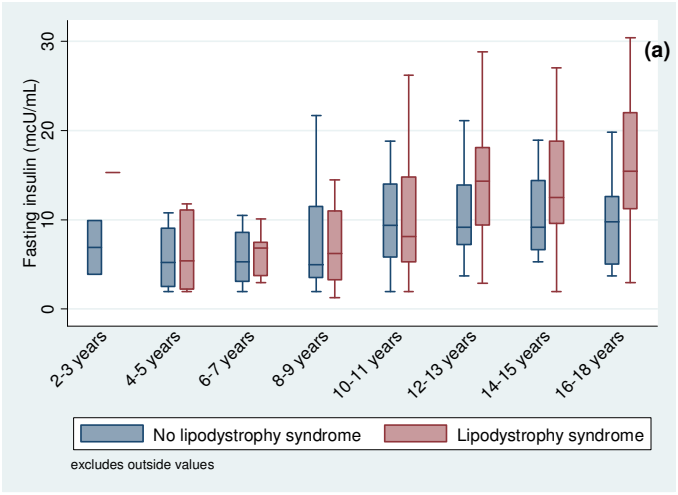
Outcome	Sex	Model	*n*	Coefficients			
				Parameter	**Estimate**	**95% confidence interval**	***p*-value**
	Female	$y_i = \alpha_i + {}^{\beta_{1i}}/_{x_i^2}$	28	α	10.84	7.94, 13.75	<0.001
				β_1	-17.82	-28.52, -7.12	0.002
		$= {}^{\beta_{2i}(ln(x_i))}/_{x_i^2}$		β_2	-17.32	-29.00, -5.64	0.005

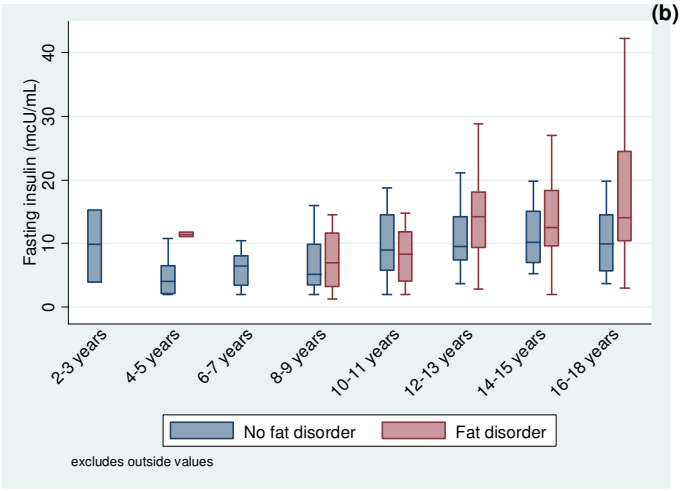
Figure C-31: Comparison of median standardized fasting insulin, stratified by sex, between subjects with/without (a) lipodystrophy syndrome, (b) fat disorder, and (c) metabolic abnormality

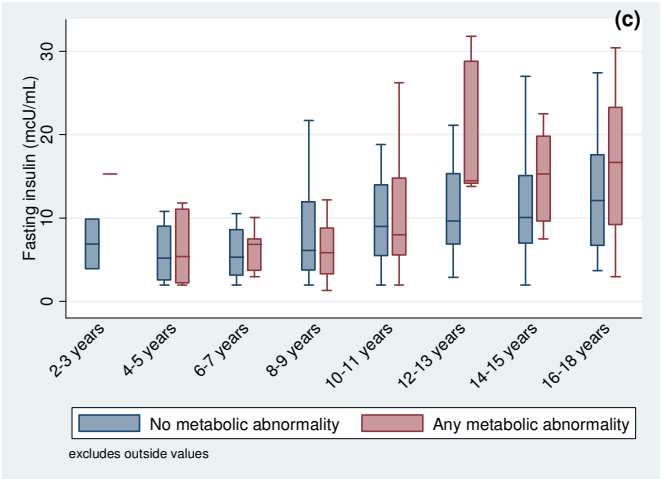


Boxes demark 25th and 75th percentiles (1st and 3rd quartile) and lines indicate adjacent values (most extreme values within 1.5 interquartile range of the nearest quartile) of the distribution within each age band. Values outside adjacent values excluded for clarity. Comparison of median standardized insulin between subjects with outcome and without outcome: $p \geq 0.057$.

Figure C-32: Comparison of median fasting insulin across age groups between subjects with/without (a) lipodystrophy syndrome, (b) fat disorder, and (c) metabolic abnormality



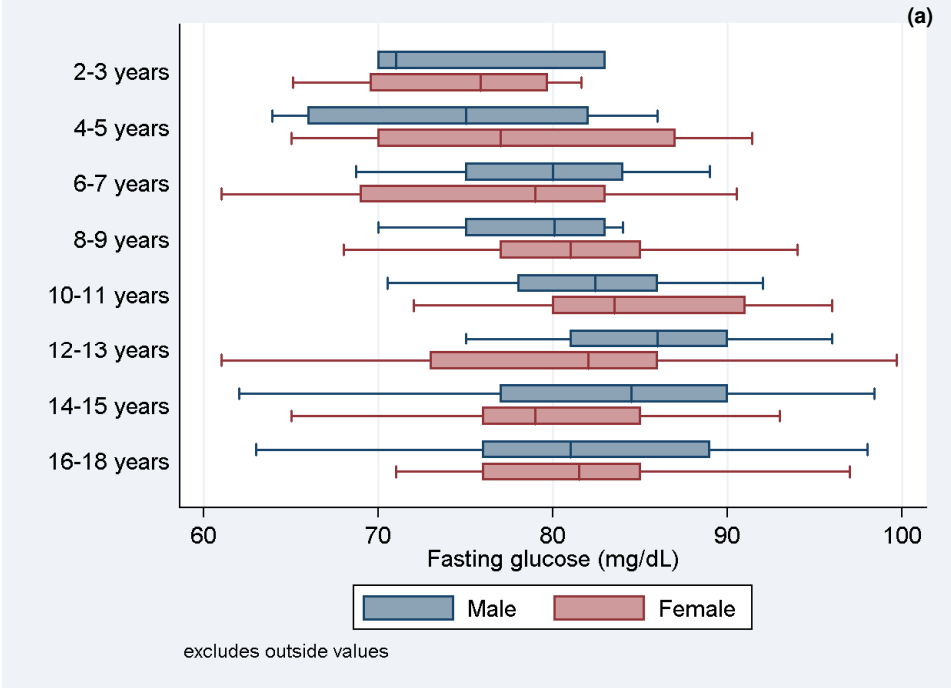




Bars indicate 25th and 75th percentiles of the distribution within each age band. No significant differences were seen in median fasting insulin between subjects with fat disorder and those without ($p > 0.05$)

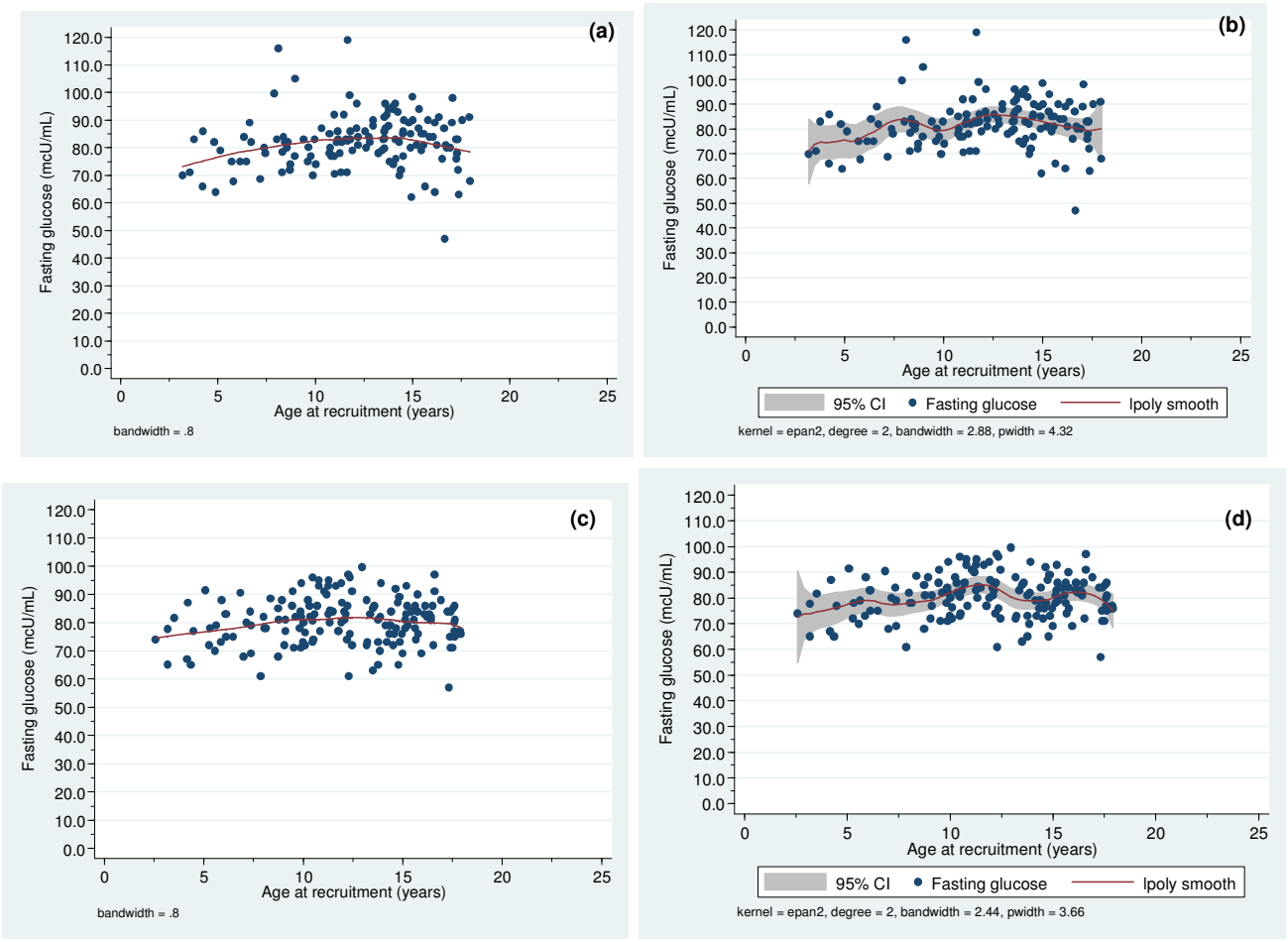
C.13 Fasting glucose

Figure C-33: Fasting glucose across age groups: (a) median fasting glucose, and (b) median standardized fasting glucose



Boxes demark 25th and 75th percentiles (2nd and 3rd quartile) and lines indicate adjacent values (most extreme values within 1.5 interquartile range of the nearest quartile) of the distribution within each age band. Values outside adjacent values excluded for clarity. No significant difference in standardized fasting glucose was seen between males and females in 2-3, 4-5, 6-7, 8-9, 12-13, 14-15 and 16-18 year olds ($p < 0.05$): significant difference seen in 10-11 year olds ($p = 0.041$)

Figure C-34: Modelling fasting glucose as a function of age at recruitment estimated: in males by (a) locally weighted smoothing, and (b) fractional polynomial model: and, in females by (locally weighted smoothing and fractional polynomial model

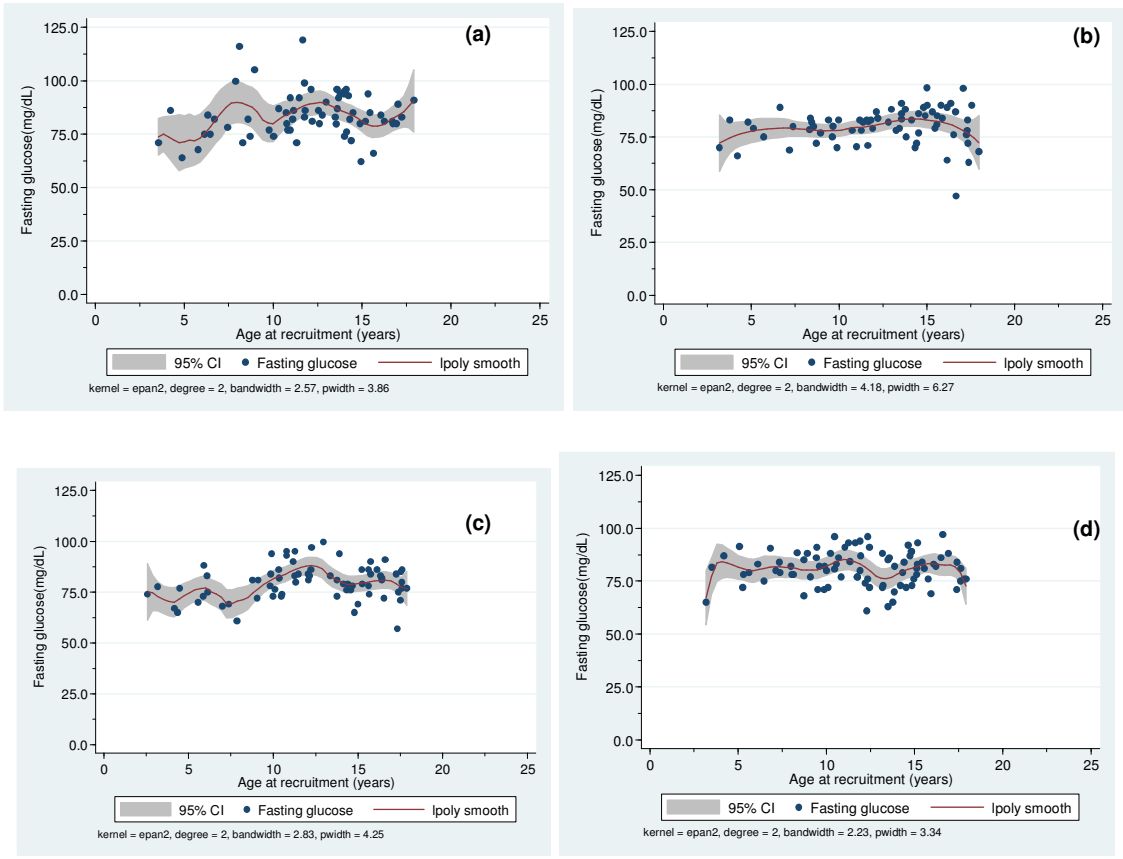


Fractional polynomial models are of order 2, estimated over 44 models. Fractional polynomial model for males: $y_i = \alpha_i + \beta_{1i}x_i^2 + \beta_i x_i^2(\ln(x_i))$ **: (*n* = 139). Fractional polynomial model for females:** $y_i = \alpha_i + \beta_{1i}x_i + \beta_{2i}x_i^2$**: (*n* = 160). Shaded area in (b) and (d) denote 95% confidence interval.**

Table C-21: Fractional polynomial models fitting fasting glucose as a function of age at recruitment, stratified by sex and lipodystrophy syndrome status

Outcome	Sex	Model	*n*	Coefficients			
				Parameter	Estimate	95% confidence interval	*p*-value
No lipodystrophy	**Male**	$y_i = \alpha_i + \beta_{1i}x_i + \beta_{2i}x_i(\ln(x_i))$	66	α	86.34	83.04, 89.65	<0.001
				β_1	47.44	6.13, 88.76	0.025
				β_2	-41.29	-79.73, -2.84	0.036
	Female	$y_i = \alpha_i + \beta_{1i}x_i^2 + \beta_{2i}x_i^3$	72	α	83.14	80.41, 85.86	<0.001
				β_1	25.83	11.46, 40.19	0.001
				β_2	-13.20	-20.78, -5.62	0.001
Lipodystrophy	**Male**	$y_i = \alpha_i + \beta_i x_i^3 + \beta_{2i}x_i^3(ln(x_1))$	71	α	82.07	79.26, 84.88	<0.001
				β_1	6.76	0.83, 12.69	0.026
			88	β_2	-10.68	-20.29, -1.06	0.030
	Female	$y_i = \alpha_i + {\beta_{1i}}/{x_i^2} + {\beta_{2i}ln(x_i)}/{x_i^2}$		α	81.16	79.40, 82.92	<0.001
				β_1	2.84	-2.42, 8.11	0.286
				β_2	3.11	-1.68, 7.90	0.200

Figure C-35: Modelling fasting glucose as a function of age at recruitment in: (a) males without lipodystrophy syndrome (*n* = 66), (b) males with lipodystrophy syndrome (*n* =71), (c) females without lipodystrophy syndrome (*n* = 72), and (d) female with lipodystrophy syndrome (*n* = 88)

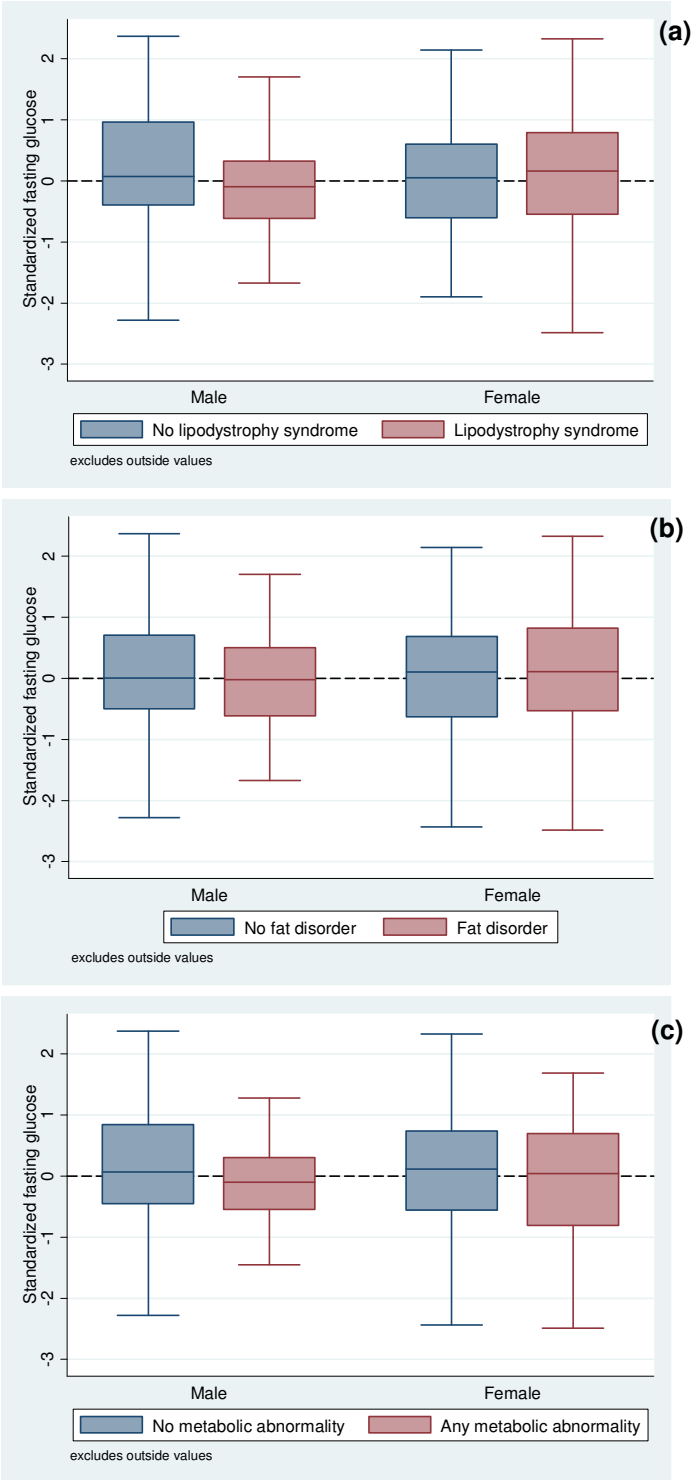


Equations: (a) $y_i = \alpha_i + \beta_{1i}x_i + \beta_{2i}x_i(\ln(x_i))$ **(b)** $y_i = \alpha_i + \beta_i x_i^3 + \beta_{2i}x_i^3(ln(x_1))$ **(c)** $y_i = \alpha_i + \beta_{1i}x_i^2 + \beta_{2i}x_i^3$ **and (d)** $y_i = \alpha_i + {\beta_{1i}}/{x_i^2} + {\beta_{2i}ln(x_i)}/{x_i^2}$ **Fractional polynomial models of 2 orders, estimated over 44 models. Shaded area denotes 95% confidence interval. See Table C-21 for details of models.**

Table C-22: Fractional polynomial models fitting fasting glucose by age at recruitment, stratified by sex metabolic abnormality status

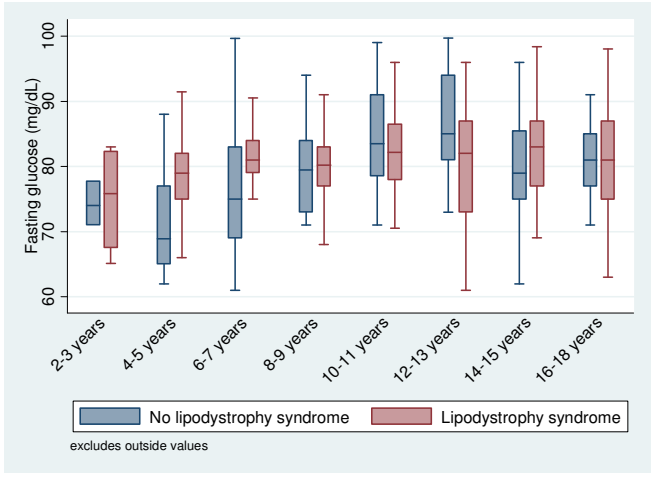
Outcome	Sex	Model	N	Coefficients			
				Parameter	Estimate	95% confidence interval	*p*-value
No metabolic abnormality	**Male**	$y_i = \alpha_i + \beta_{1i}\sqrt[2]{x_i} + \beta_{2i}x_i^2$	94	α	85.33	82.59, 88.06	<0.001
				β_1	65.48	15.95, 115.01	0.010
				β_2	-13.41	-23.70, -3.13	0.011
	Female	$y_i = \alpha_i + \beta_{1i}x_i^2 + \beta_{2i}x_i^2(ln(x_i))$	116	α	83.13	81.12, 85.15	<0.01
				β_1	12.85	5.25, 20.45	0.001
				β_2	-17.77	-28.31, -7.23	0.001
Metabolic abnormality	**Male**	$y_i = \alpha_i + \beta_{1i}x_i^3 + \beta_{2i}x_i^3(ln(x_i))$	43	α	80.81	77.93, 83.68	<0.001
				β_1	5.42	-0.49, 11.33	0.071
				β_2	-7.95	-17.83, 1.93	0.112
	Female	$y_i = \alpha_i + \beta_{1i}/x_i^2 = \beta_{2i}(ln(x_i))/x_i^2$	44	α	79.93	77.23, 82.63	<0.001
				β_1	3.24	-4.10, 10.60	0.378
				β_2	3.31	-3.07, 9.70	0.301

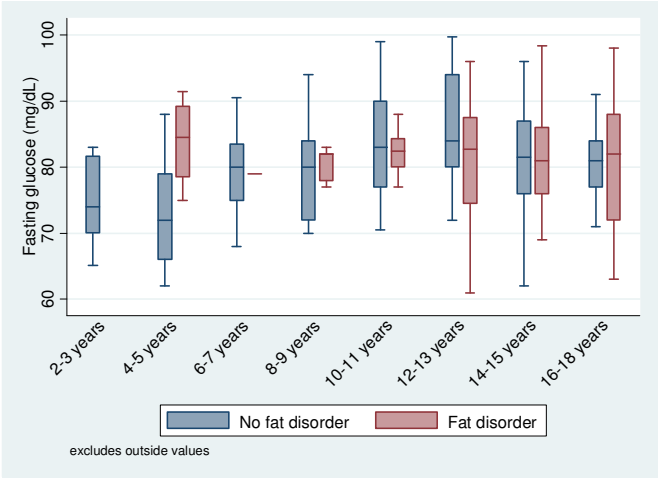
Figure C-36: Comparison of median standardized fasting glucose, stratified by sex, between subjects with/without (a) lipodystrophy syndrome, (b) fat disorder, and (c) metabolic abnormality

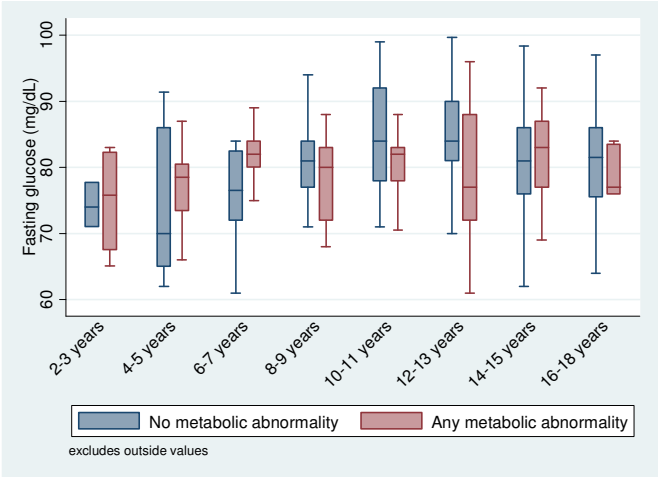


Boxes demark 25th and 75th percentiles (1st and 3rd quartile) and lines indicate adjacent values (most extreme values within 1.5 interquartile range of the nearest quartile) of the distribution within each age band. Values outside adjacent values excluded for clarity. Comparison of median standardized fasting glucose between subjects with outcome and without outcome: $p \geq 0.157$.

Figure C-37: Comparison of median fasting glucose across age groups between subjects with/without (a) lipodystrophy syndrome, (b) fat disorder, and (c) metabolic abnormality







Bars indicate 25th and 75th percentiles of the distribution within each age band. No significant differences were seen in median fasting glucose between subjects with fat disorder and those without ($p > 0.05$)

Appendix D Appendix to chapter 4

D.1 Prevalence of body fat alterations outcomes by categories of antiretroviral therapy

Table D-1: Prevalence of fat alteration outcomes by categories of antiretroviral therapy: exclusion of ART-naïve/ART status missing subjects

		Body fat alterations			Lipohypertrophy			Lipoatrophy			Both lipohypertrophy and lipoatrophy		
		Prevalence	n/N	*p*-value	Prevalence	n/N	*p*-value	Prevalence	n/N	*p*-value	Prevalence	n/N	*p*-value
NRTI	**Not current**	50.00	1/2	0.868	50.00	1/2	0.487	50.00	1/2	0.549	50.00	1/2	0.143
	Current	44.17	159/360		27.86	100/359		30.45	109/358		13.89	50/360	
NNRTI	**Not current**	41.56	96/231	0.179	25.65	59/230	0.192	27.95	64/229	0.156	11.69	27/231	0.081
	Current	48.85	64/131		32.06	42/131		35.11	46/131		18.32	24/131	
PI	**Not current**	44.83	65/145	0.844	26.90	39/145	0.708	30.34	44/145	0.943	15.21	18/145	0.454
	Current	43.78	95/217			62/216		30.74	66/215		14.09	51/362	

Association investigated using χ^2 test

D.2 Univariable analyses for body fat alterations

Table D-2: Univariable analysis for fat alterations outcomes by participant characteristic at recruitment

		Body fat alterations			Lipohypertrophy			Lipoatrophy			Combined lipohypertrophy and lipoatrophy		
		OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Demographic factors													
Gender	**Male**	1			1			1			1		
	Female	1.24	(0.84, 1.84)	0.287	1.76	(1.13, 2.75)	0.012	0.87	(0.56, 1.35)	0.537	1.31	(0.74, 2.34)	0.359
Age (years)	**2-6**	1			1			1			1		
	7-11	2.92	(1.19, 7.13)	0.019	2.53	(0.91, 7.04)	0.075	3.75	(1.07, 13.13)	0.039	4.85	(0.61, 38.45)	0.135
	12-18	4.80	(2.06, 11.18)	<0.001	3.40	(1.29, 8.96)	0.013	6.82	(2.05, 22.66)	0.002	8.21	(1.10, 61.26)	0.040
Ethnicity	**Black**	1			1			1			1		
	White	3.29	(1.97, 5.48)	<0.001	2.34	(1.33, 4.12)	0.003	4.93	(2.46, 9.89)	<0.001	5.30	(1.86, 15.10)	0.002
	Other	1.44	(0.46, 4.50)	0.529	1.52	(0.44, 5.20)	0.504	1.28	(0.26, 6.42)	0.764	1.61	(0.17, 15.33)	0.679
Country of residence	**Italy**	1			1			1					
	Belgium	0.46	(0.27, 0.80)	0.005	0.61	(0.34, 1.13)	0.116	0.42	(0.21, 0.84)	0.014	0.55	(0.21, 1.48)	0.236
	Poland	1.57	(0.91, 2.69)	0.104	1.49	(0.85, 2.63)	0.166	3.16	(1.82, 5.51)	<0.001	4.66	(2.46, 8.84)	<0.001
Infection factors													
CDC immune stage at recruitment	**Stage 1**	1			1			1			1		
	Stage 2	0.51	(0.31, 0.86)	0.012	0.66	(0.37, 1.16)	0.150	0.44	(0.24, 0.83)	0.010	0.52	(0.22, 1.19)	0.120
	Stage 3	0.66	(0.24,	0.428	0.52	(0.14,	0.306	0.68	(0.22,	0.515	0.36	(0.05,	0.321

		Body fat alterations			Lipohypertrophy			Lipoatrophy			Combined lipohypertrophy and lipoatrophy		
		OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
			1.83)			1.83)			2.15)			2.74)	
Nadir CDC immune stage	**Stage 1**	1			1			1			1		
	Stage 2	0.80	(0.52, 1.24)	0.314	1.28	(0.79, 2.08)	0.313	0.63	(0.39, 1.03)	0.063	1.13	(0.60, 2.12)	0.710
	Stage 3	1.21	(0.71, 2.04)	0.487	1.10	(0.61, 2.01)	0.745	1.15	(0.66, 2.02)	0.621	1.01	(0.46, 2.22)	0.976
CDC clinical stage at recruitment	**N+A**	1			1			1			1		
	B	1.32	(0.55, 3.20)	0.533	1.11	(0.42, 2.94)	0.837	1.73	(0.69, 4.31)	0.239	1.74	(0.56, 5.42)	0.340
	C	0.55	(0.14, 2.10)	0.378	1.04	(0.27, 4.00)	0.956	0.62	(0.13, 2.95)	0.552	1.64	(0.34, 7.87)	0.535
Maximum CDC clinical stage	**N+A**	1			1			1			1		
	B	2.35	(1.48, 3.73)	<0.001	2.09	(1.25, 3.49)	0.005	2.15	(1.28, 3.61)	0.004	2.18	(1.10, 4.31)	0.026
	C	2.48	(1.45, 4.23)	0.001	2.09	(1.17, 3.73)	0.013	2.16	(1.20, 3.90)	0.010	1.96	(0.90, 4.29)	0.089
Viral load (copies/ml)	**≤50**	1			1			1			1		
	>50	0.98	(0.56, 1.72)	0.942	0.86	(0.47, 1.59)	0.633	0.97	(0.52, 1.80)	0.917	0.79	(0..37, 1.72)	0.558
Maximum duration of ART use	**Per year**	1.04	(0.98, 1.10)	0.187	1.01	(0.96, 1.08)	0.620	1.04	(0.98, 1.10)	0.239	1.01	(0.94, 1.09)	0.782
Other factors													
Hepatitis C co-infection	**Uninfected**	1			1			1			1		
	Infected	2.96	(1.24, 7.07)	0.015	2.80	(1.22, 6.44)	0.015	1.59	(0.68, 3.74)	0.288	1.74	(0.62, 4.88)	0.389
Tanner score for puberty	**I**	1			1			1			1		
	II-IV	2.43	(1.47, 4.01)	0.001	1.75	(1.00, 3.08)	0.052	2.54	(1.44, 4.47)	0.001	1.93	(0.92, 4.06)	0.082
	V	2.75	(1.58, 4.79)	<0.001	2.49	(1.36, 4.56)	0.003	2.05	(1.09, 3.84)	0.026	1.95	(0.87, 4.37)	0.107
BMI	**Kg/m2**	1.14	(1.08, 1.21)	<0.001	1.28	(1.19, 1.37)	<0.001	0.97	(0.91, 1.03)	0.323	-	-	-
NRTI													
Any NRTI	**Not current**	1			1			1			1		
	Current	2.60	(1.09,	0.032	1.54	(0.61,	0.356	3.94	(1.17,	0.027	2.26	(0.52,	0.276

		Body fat alterations			Lipohypertrophy			Lipoatrophy			Combined lipohypertrophy and lipoatrophy		
		OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
			6.21)			3.89)			13.26)			9.77)	
Didanosine	**Not current**	1			1			1			1		
	Current	1.00	(0.54, 1.85)	0.999	1.06	(0.53, 2.10)	0.870	1.05	(0.54, 2.05)	0.882	1.16	(0.49, 2.74)	0.737
Lamivudine	**Not current**	1			1			1			1		
	Current	0.86	(0.56, 1.28)	0.432	0.73	(0.46, 1.15)	0.175	0.75	(0.48, 1.17)	0.208	0.49	(0.27, 0.88)	0.018
Tenofovir	**Not current**	1			1			1			1		
	Current	1.55	(0.98, 2.43)	0.059	1.56	(0.96, 2.54)	0.075	1.57	(0.97, 2.54)	0.066	1.92	(1.04, 3.54)	0.036
Stavudine	**Not current**	1			1			1			1		
	Current	3.31	(1.69, 6.47)	<0.001	1.81	(0.94, 3.48)	0.075	2.66	(1.40, 5.07)	0.003	1.53	(0.67, 3.49)	0.318
Zidovudine	**Not current**	1			1			1			1		
	Current	0.60	(0.38, 0.97)	0.036	0.58	(0.33, 1.00)	0.052	0.53	(0.31, 0.92)	0.025	0.33	(0.14, 0.81)	0.015
NNRTI													
Any NNRTI	**Not current**	1			1			1			1		
	Current	1.47	(0.96, 2.25)	0.075	1.43	(0.90, 2.27)	0.130	1.57	(0.99, 2.47)	0.053	1.85	(1.02, 3.34)	0.041
Efavirenz	**Not current**	1			1			1			1		
	Current	1.71	(1.07, 2.74)	0.024	1.68	(1.02, 2.77)	0.042	1.82	(1.11, 2.98)	0.017	2.27	(1.23, 4.20)	0.009
PI													
Any PI	**Not current**	1			1			1			1		
	Current	1.12	(0.75, 1.68)	0.587	1.18	(0.75, 1.85)	0.472	1.22	(0.78, 1.91)	0.375	1.45	(0.79, 2.66)	0.225
Type of drug therapy at recruitment	**PI-based HAART**	1			1			1			1		
	NRTI mono-therapy	0.84	(0.38, 1.87)	0.666	0.56	(0.20, 1.54)	0.262	0.79	(0.32, 1.95)	0.609	0.23	(0.03, 1.79)	0.162
	NNRTI-based HAART	1.20	(0.76, 1.89)	0.446	1.12	(0.67, 1.85)	0.671	1.16	(0.71, 1.91)	0.551	1.13	(0.58, 2.17)	0.723

	Body fat alterations			Lipohypertrophy			Lipoatrophy			Combined lipohypertrophy and lipoatrophy		
	OR	**95% CI**	***p*-value**	**OR**	**95% CI**	***p*-value**	**OR**	**95% CI**	***p*-value**	**OR**	**95% CI**	***p*-value**
Triple class	3.09	(0.92, 10.35)	0.068	3.14	(1.01, 9.75)	0.048	3.97	(1.25, 12.65)	0.020	5.62	(1.76, 17.98)	0.004

Odds ratios (OR) and accompanying 95% confidence interval (95% CI) for fat alterations by participant characteristic. CDC-defined immune status - stage 1: no immunosuppression, stage 2: moderate immunosuppression, stage 3: severe immunosuppression. CDC-defined clinical status - N + A: no symptoms/asymptomatic, B: moderate symptoms, C: severe symptoms.

Table D-3: Univariable analysis for body fat alterations outcomes by ever use of antiretroviral therapy

		Body fat alterations *n*=422			Lipohypertrophy *n*=421			Lipoatrophy *n*=420			Both lipoatrophy and lipohypertrophy *n*=422		
		OR	(95% CI)	*p*	OR	(95% CI)	*p*	OR	(95% CI)	*p*	OR	(95% CI)	*p*
Ever use of ART	**No**	1			1			1			1		
	Yes	2.78	(1.10, 7.01)	0.03	1.79	(0.66, 4.82)	0.251	5.4	(1.26, 23.11)	0.023	4.38	(0.58, 32.90)	0.151
Any NRTI	**No**	1			1			1			1		
	Yes	2.78	(1.10, 7.01)	0.03	1.79	(0.66, 4.82)	0.251	5.4	(1.26, 23.11)	0.023	4.38	(0.58, 32.90)	0.151
Didanosine	**No**	1			1			1			1		
	Yes	2.74	(1.83, 4.07)	<0.001	2.49	(1.60, 3.87)	<0.001	2.69	(1.73, 4.18)	<0.001	3.37	(1.82, 6.23)	<0.001
Lamivudine	**No**	1			1			1			1		
	Yes	1.60	(0.84, 3.06)	0.151	1.26	(0.62, 2.57)	0.524	1.49	(0.71, 3.10)	0.288	1.05	(0.42, 2.60)	0.914
Stavudine	**No**	1			1			1			1		
	Yes	3.92	(2.59, 5.93)	<0.001	2.43	(1.55, 3.81)	<0.001	4.98	(3.05, 8.15)	<0.001	4.09	(2.09, 8.00)	<0.001
Zidovudine	**No**	1			1			1			1		
	Yes	1.16	(0.75, 1.79)	0.511	1.03	(0.63, 1.66)	0.919	1.13	(0.70, 1.84)	0.619	0.93	(0.50, 1.73)	0.812
Tenofovir	**No**	1			1			1			1		
	Yes	1.62	(1.06, 2.47)	0.025	1.52	(0.96, 2.40)	0.071	2.01	(1.28, 3.15)	0.002	2.51	(1.42, 4.45)	0.002
Any NNRTI	**No**	1			1			1			1		
	Yes	1.81	(1.21, 2.70)	0.004	1.56	(1.00, 2.43)	0.05	1.96	(1.25, 3.08)	0.004	2.01	(1.08, 3.71)	0.026
Efavirenz	**No**	1			1			1			1		
	Yes	2.51	(1.68, 3.77)	<0.001	2.02	(1.31, 3.13)	0.002	2.55	(1.65, 3.95)	<0.001	2.54	(1.43, 4.50)	0.001
Nevirapine	**No**	1			1			1			1		
	Yes	0.93	(0.59, 1.46)	0.753	1.11	(0.68, 1.81)	0.687	0.88	(0.53, 1.45)	0.619	1.14	(0.60, 2.15)	0.690
Any PI	**No**	1			1			1			1		
	Yes	2.11	(1.28, 3.46)	0.003	2.15	(1.20, 3.87)	0.01	2.23	(1.24, 4.00)	0.007	3.33	(1.29, 8.59)	0.013
Ritonavir booster	**No**	1			1			1			1		
	Yes	1.59	(1.06, 2.38)	0.024	1.81	(1.14, 2.86)	0.012	1.76	(1.12, 2.78)	0.015	3.01	(1.51, 6.00)	0.002
Indinavir	**No**	1			1			1			1		

		Body fat alterations *n*=422			Lipohypertrophy *n*=421			Lipoatrophy *n*=420			Both lipoatrophy and lipohypertrophy *n*=422		
		OR	**(95% CI)**	***p***	**OR**	**(95% CI)**	***p***	**OR**	**(95% CI)**	***p***	**OR**	**(95% CI)**	***p***
	Yes	4.13	(1.70, 10.06)	0.002	2.87	(1.29, 6.40)	0.010	3.3	(1.48, 7.36)	0.004	3.22	(1.33, 7.81)	0.010
Nelfinavir	**No**	1			1			1			1		
	Yes	1.83	(1.24, 2.70)	0.003	1.51	(0.98, 2.32)	0.062	1.39	(0.91, 2.14)	0.128	1.01	(0.57, 1.76)	0.986
Saquinavir	**No**	1			1			1			1		
	Yes	2.5	(0.96, 6.48)	0.06	3.94	(1.54, 10.06)	0.004	1.54	(0.59, 4.02)	0.375	3.26	(1.18, 8.96)	0.022

D.3 Multivariable analyses for body fat alterations

See next page.

Table D-4: Final multivariable models for body fat alteration outcomes with ever-use of specific antiretroviral drugs as explanatory variables

		Body fat alterations *n*=342			Any lipohypertrophy *n*=354			Any lipoatrophy *n*=344			Combined lipohypertrophy and lipoatrophy *n*=359		
		n	**AOR (95% CI)**	***P***	***n***	**AOR (95% CI)**	***p***	***n***	**AOR (95% CI)**	***p***	***n***	**AOR (95% CI)**	***p***
Age	**per year**	342	1.03 (0.95, 1.12)	0.491	354	0.93 (0.85, 1.02)	0.137	344	1.17 (1.06, 1.28)	0.001	359	1.13 (1.00, 1.27)	0.045
Duration of ART	**per year**	342	0.98 (0.91, 1.05)	0.608	354	0.97 (0.90, 1.05)	0.496	344	0.98 (0.91, 1.06)	0.65	359	0.95 (0.86, 1.04)	0.270
Ethnicity	**Black**	93	1					92	1				
	White	232	2.56 (1.25, 5.23)	0.01				235	3.87 (1.50, 10.01)	0.005			
	Other	17	1.08 (0.28, 4.19)	0.913				17	0.97 (0.16, 6.03)	0.978			
BMI (kg/m^2)	**per unit**	342	1.2 (1.09, 1.31)	<0.001	354	1.46 (1.30, 1.64)	<0.001						
Stavudine	**Never**	156	1		161	1		157	1				
	Ever	186	3.56 (2.08, 6.09)	<0.001	193	2.53 (1.38, 4.63)	0.003	187	4.98 (2.58, 9.61)	<0.001			
Efavirenz	**Never**	212	1					212	1		222	1	
	Ever	130	1.86 (1.10, 3.16)	0.022				132	1.87 (1.05, 3.35)	0.034	137	2.38 (1.11, 5.12)	0.026
Didanosine	**Never**										179	1	
	Ever										180	3.44 (1.47, 8.07)	0.004
Ritonavir booster	**Never**										128	1	
	Ever										231	2.92 (1.16, 7.33)	0.023
Indinavir	**Never**										338	1	
	Ever										21	4 (1.04, 15.41)	0.044

Models constructed using reverse stepwise logistic regression. CDC-defined immune status - stage 1: no immunosuppression, stage 2: moderate immunosuppression, stage 3: severe immunosuppression. CDC-defined clinical status - N + A: no symptoms/asymptomatic, B: moderate symptoms, C: severe symptoms.

Table D-5: Sensitivity analysis: Multivariable models for moderate/severe body fat alteration outcomes

		Body fat alteration *n*=302			Any lipohypertrophy *n*=338			Any lipoatrophy *n*=305			Combined lipohypertrophy and lipoatrophy *n*=97		
		n	**AOR (95% CI)**	***p***	***n***	**AOR (95% CI)**	***p***	***n***	**AOR (95% CI)**	***p***	***n***	**AOR (95% CI)**	***p***
Age	**per year**	302	1.09 (0.97, 1.22)	0.158	338	0.99 (0.86, 1.14)	0.895	305	1.24 (1.09, 1.42)	0.001	97	0.85 (0.60, 1.21)	0.371
Duration of ART	**per year**	302	1.05 (0.96, 1.15)	0.280	338	1.03 (0.92, 1.14)	0.634	305	1.07 (0.97, 1.18)	0.191	97	1.15 (0.89, 1.49)	0.275
Ethnicity	**Black**	73	1					73	1		50	1	
	White	215	3.17 (1.09, 9.28)	0.035				218	3.17 (0.89, 11.39)	0.076	44	20.19 (1.40, 291.36)	0.027
	Other	14	3.81 (0.64, 22.87)	0.143				14	-	0.997	3	-	1.00
Maximum CDC clinical status	**N+A**	121	1		139	1		121	1				
	B	110	0.88 (0.40, 1.92)	0.747	122	0.81 (0.32, 2.08)	0.663	111	1.11 (0.46, 2.68)	0.808			
	C	71	1.54 (0.68, 3.48)	0.300	77	1.22 (0.43, 3.43)	0.707	73	1.53 (0.57, 4.06)	0.398			
Nadir CDC Immune status	**Stage 1**							111	1				
	Stage 2							123	0.32 (0.14, 0.76)	0.010			
	Stage 3							71	0.40 (0.14, 1.08)	0.071			
BMI (kg/m^2)	**per unit**	302	1.21 (1.09, 1.35)	<0.001	338	1.51 (1.29, 1.75)	<0.001				97	1.16 (0.83, 1.61)	0.397
Efavirenz	**Never**	226	1										
	Ever	41	0.82 (0.31, 2.18)	0.698									
Didanosine	**Never**	261	1										
	Ever	41	0.82 (0.31, 2.18)	0.698									
Lamivudine	**Never**										25	1	
	Ever										72	0.06 (0.01, 0.58)	0.015
Stavudine	**Never**	266	1					269	1				

		Body fat alteration *n*=302			Any lipohypertrophy *n*=338			Any lipoatrophy *n*=305			Combined lipohypertrophy and lipoatrophy *n*=97		
		n	**AOR (95% CI)**	***p***	***n***	**AOR (95% CI)**	***p***	***n***	**AOR (95% CI)**	***p***	***n***	**AOR (95% CI)**	***p***
Zidovudine	**Ever**	36	1.71 (0.65, 4.55)	0.279				36	2.18 (0.77, 6.15)	0.141			
	Never										53	1	
	Ever										44	-	1.00

Model structure defined by backward stepwise regression using fat disorder outcomes as outcome variables: optimal models then repeated using moderate/severe fat disorder as the outcome variables. CDC-defined immune status - stage 1: no immunosuppression, stage 2: moderate immunosuppression, stage 3: severe immunosuppression. CDC-defined clinical status - N + A: no symptoms/asymptomatic, B: moderate symptoms, C: severe symptoms.

Table D-6: Final multivariable models for body fat alteration outcome adjusted by Tanner score for puberty

		Body fat alteration *n*=268			Any lipohypertrophy *n*=300			Any lipoatrophy *n*=2667			Combined lipohypertrophy and lipoatrophy *n*=283		
		n	**AOR (95% CI)**	***p***	***n***	**AOR (95% CI)**	***p***	***n***	**AOR (95% CI)**	***p***	***n***	**AOR (95% CI)**	***p***
Age	**per year**	268	1.03 (0.89, 1.20)	0.656	300	0.95 (0.82, 1.10)	0.491	267	1.14 (0.97, 1.33)	0.114	283	1.01 (0.82, 1.24)	0.922
Duration of ART	**per year**	268	1.07 (0.98, 1.17)	0.115	300	1.00 (0.92, 1.09)	0.987	267	1.05 (0.96, 1.16)	0.263	283	1.02 (0.91, 1.15)	0.721
Tanner score for puberty	**I**	97	1		111	1		96	1		101	1	
	II-IV	106	1.37 (0.51, 3.71)	0.531	114	0.95 (0.34, 2.69)	0.922	106	1.81 (0.62, 5.33)	0.280	114	1.39 (0.35, 5.56)	0.646
	V	65	1.36 (0.34, 5.37)	0.663	75	0.75 (0.18, 3.06)	0.685	65	1.82 (0.41, 7.98)	0.429	68	1.44 (0.22, 9.31)	0.701
Ethnicity	**Black**	64	1					63	1		73	1	
	White	190	2.89 (1.21, 6.91)	0.017				190	4.18 (1.43, 12.26)	0.009	195	3.15 (0.71, 14.00)	0.132
	Other	14	1.72 (0.38, 7.85)	0.484				14	1.37 (0.21, 9.06)	0.743	15	0.82 (0.06, 10.87)	0.879
Maximum CDC clinical status	**N+A**	111	1		128	1		110	1				
	B	94	1.72 (0.86, 3.42)	0.125	104	2.17 (1.07, 4.43)	0.033	94	1.98 (0.94, 4.16)	0.073			
	C	63	2.72 (1.29, 5.72)	0.009	68	2.50 (1.13, 5.51)	0.023	63	1.87 (0.80, 4.36)	0.146			
Nadir CDC Immune status	**Stage 1**							99	1				
	Stage 2							109	0.44 (0.21, 0.93)	0.030			
	Stage 3							59	0.69 (0.29, 1.68)	0.414			
BMI (kg/m^2)	**per unit**	268	1.17 (1.06, 1.30)	0.003	300	1.46 (1.29, 1.65)	<0.001				283	1.20 (1.05, 1.37)	0.006
Efavirenz	**Never**	200	1										
	Ever	68	2.39 (1.19, 4.79)	0.014									
Didanosine	**Never**	231	1										
	Ever	37	0.46 (0.18, 1.18)	0.105									
Lamivudine	**Never**										88	1	
	Ever										195	0.52 (0.23, 1.19)	0.120

		Body fat alteration *n*=268			Any lipohypertrophy *n*=300			Any lipoatrophy *n*=2667			Combined lipohypertrophy and lipoatrophy *n*=283		
		n	**AOR (95% CI)**	***p***	***n***	**AOR (95% CI)**	***p***	***n***	**AOR (95% CI)**	***p***	***n***	**AOR (95% CI)**	***p***
Stavudine	**Never**	234	1					234	1				
	Ever	34	5.01 (1.92, 13.05)	0.001				33	2.19 (0.87, 5.53)	0.098			
Zidovudine	**Never**										202	1	
	Ever										81	0.32 (0.10, 0.98)	0.045

Model structure defined by backward stepwise regression using fat disorder outcomes as outcome variables , with addition of Tanner score for puberty as an additional variable. CDC-defined immune status - stage 1: no immunosuppression, stage 2: moderate immunosuppression, stage 3: severe immunosuppression. CDC-defined clinical status - N + A: no symptoms/asymptomatic, B: moderate symptoms, C: severe symptoms.

Table D-7: Final multivariable models for body fat alteration outcome with the addition of an interaction between sex and age

		Body fat alteration *n*=296			Any lipohypertrophy *n*=330			Any lipoatrophy *n*=297			Combined lipohypertrophy and lipoatrophy *n*=310		
		n	**AOR (95% CI)**	***p***	***n***	**AOR (95% CI)**	***p***	***n***	**AOR (95% CI)**	***p***	***n***	**AOR (95% CI)**	***p***
Age	**per year**	296	1.07 (0.95, 1.20)	0.241	330	0.93 (0.83, 1.05)	0.237	297	1.21 (1.06, 1.38)	0.004	310	1.08 (0.90, 1.30)	0.400
Duration of ART	**per year**	296	1.05 (0.97, 1.13)	0.257	330	0.99 (0.92, 1.07)	0.817	297	1.04 (0.96, 1.13)	0.361	310	1.03 (0.92, 1.14)	0.645
Sex	**Male**	153	1		168	1		155	1		160	1	
	Female	143	1.34 (0.20, 9.12)	0.766	162	1.18 (0.17, 8.03)	0.863	142	2.19 (0.25, 19.17)	0.478	150	4.57 (0.23, 90.24)	0.318
Age*Female Interaction		143	1.02 (0.87, 1.18)	0.845	162	1.01 (0.87, 1.18)	0.856	142	0.98 (0.83, 1.15)	0.780	150	0.94 (0.75, 1.17)	0.563
Ethnicity	**Black**	73	1					72	1		81	1	
	White	209	3.17 (1.43, 7.02)	0.004				211	4.05 (1.56, 10.52)	0.004	214	4.16 (0.99, 17.57)	0.052
	Other	14	1.98 (0.47, 8.37)	0.354				14	1.34 (0.21, 8.30)	0.756	15	0.77 (0.06, 10.34)	0.845
Maximum CDC clinical status	**N+A**	121	1		139	1		120	1				
	B	108	1.85 (0.98, 3.49)	0.059	118	2.16 (1.11, 4.20)	0.024	109	1.95 (0.98, 3.87)	0.056			
	C	67	2.34 (1.16, 4.71)	0.018	73	2.17 (1.03, 4.59)	0.043	68	1.70 (0.76, 3.79)	0.197			
Nadir CDC Immune status	**Stage 1**							109	1				
	Stage 2							120	0.37 (0.18, 0.74)	0.005			
	Stage 3							68	0.62 (0.27, 1.39)	0.243			
BMI (kg/m^2)	**per unit**	296	1.15 (1.05, 1.27)	0.004	330	1.43 (1.28, 1.61)	<0.001				310	1.20 (1.06, 1.37)	0.005
Efavirenz	**Never**	222	1										
	Ever	74	1.87 (0.99, 3.52)	0.053									
Didanosine	**Never**	255	1										
	Ever	41	0.46 (0.19, 1.10)	<0.001									
Lamivudine	**Never**										97	1	
	Ever										213	0.36 (0.15, 0.83)	0.017

		Body fat alteration *n*=296			Any lipohypertrophy *n*=330			Any lipoatrophy *n*=297			Combined lipohypertrophy and lipoatrophy *n*=310		
		n	**AOR (95% CI)**	***p***	***n***	**AOR (95% CI)**	***p***	***n***	**AOR (95% CI)**	***p***	***n***	**AOR (95% CI)**	***p***
Stavudine	**Never**	260	1					262	1				
	Ever	36	5.66 (2.25, 14.28)	<0.001				35	2.78 (1.15, 6.70)	0.023			
Zidovudine	**Never**										217	1	
	Ever										93	0.38 (0.14, 1.08)	0.070

Model structure defined by backward stepwise regression using fat disorder outcomes as outcome variables , with addition of an interaction for sex and age. CDC-defined immune status - stage 1: no immunosuppression, stage 2: moderate immunosuppression, stage 3: severe immunosuppression. CDC-defined clinical status - N + A: no symptoms/asymptomatic, B: moderate symptoms, C: severe symptoms.

Table D-8: Multivariable logistic regressions for body fat alterations including categories of ART as explanatory variables: threshold for stepwise covariate selection set at 10% significance

		Body fat alterations *n* = 302	Lipohypertrophy *n* = 301	Lipoatrophy *n* = 303	Both lipohypertrophy and lipoatrophy *n* = 320
Age	**Per year**	1.06 (0.97, 1.16)	0.93 (0.84, 1.03)	1.16 (1.06, 1.27)***	1.17 (1.04, 1.31)**
Ethnicity	**Black**	1	1	1	1
	White	3,58 (1.59, 7.62)**	2.17 (0.88, 5.36) †	5.08 (1.95, 13.27)***	5.22 (1.36, 20.03)***
	Other	1.82 (0.44, 7.55)	1.44 (0.27, 7.71)	1.62 (0.27, 9.72)	0.72 (0.05, 9.91)
Body mass index	**Per kg/m^2**	1.17 (1.07, 1.29)***	1.45 (1.28, 1.63)***		
Maximum CDC clinical status	**N/A**	1	1	1	
	B	1.94 (1.05, 3.56)*	2.06 (1.00, 4.26)†	1.78 (0.92, 3.45)†	
	C	2.59 (1.32, 5.11)**	2.86 (1.31, 6.25)**	1.71 (0.82, 3.57)	
Duration of ART	**Per year**	1.06 (0.97, 1.16)	1.02 (0.94, 1.11)	1.03 (0.95, 1.11)	1.01 (0.91, 1.11)
NNRTI use at recruitment	**No**	1	1	1	1
	Yes	1.97 (1.11, 3.50)*	1.77 (0.92, 3.41)†	1.85 (1.01, 3.42)*	8.18 (2.18, 30.63)**
PI use at recruitment	**No**				1
	Yes				6.55 (1.73, 24.74)**

†0.10 > p ≥ 0.05, *0.05 > p ≥> 0.01, **0.01 > p ≥ 0.001, ***0.001< p

Table D-9: Multivariable logistic regressions for body fat alterations including specific ART drugs as explanatory variables: threshold for stepwise covariate selection set at 10% significance

		Body fat alterations *n* = 302	Lipohypertrophy *n* = 337	Lipoatrophy *n* = 318	Both lipohypertrophy and lipoatrophy *n* = 305
Age	**Per year**	1.08 (0.99, 1.18)	0.93 (0.85, 1.02)	1.20 (1.10, 1.31)***	1.14 (1.02, 1.28)*
Ethnicity	**Black**	1		1	1
	White	2.90 (1.36, 6.17)**		4.81)1.90, 12.16)***	3.45 (0.87, 13.62)†
	Other	1.73 (0.42, 7.12)		1.47 (0.25, 8.69)	1.25 (0.11, 14.68)
Body mass index	**Kg/m^2**	1.16 (1.05, 1.27)**	1.46 (1.30, 1.63)***		
Maximum CDC-defined clinical symptoms	**N/A**	1	1		1
	B	1.81 (0.97, 3.38)†	2.08 (1.07, 4.05)*		2.29 (0.93, 5.61)†
	C	2.36 (1.20, 4.66)*	2.24 (1.07, 4.69)*		1.80 (0.68, 4.77)
Duration of ART	**Per year**	1.04 (0.96, 1.12)	0.99 (0.91, 1.07)	1.03 (0.96, 1.12)	0.99 (0.89, 1.09)
Zidovudine at recruitment	**No**				1
	Yes				0.28 (0.10, 0.78)*
Stavudine at recruitment	**No**	1		1	
	Yes	4.59 (1.88, 11.25)***		3.19 (1.36, 7.47)**	
Efavirenz at recruitment	**No**	1		1	
	Yes	1.99 (1.07, 3.68)*		1.73 (0.91, 3.27)†	

†0.10 > p ≥ 0.05, *0.05 > p ≥> 0.01, **0.01 > p ≥ 0.001, ***0.001< p

Appendix E Appendix to Chapter 5

E.1 Prevalence of metabolic abnormality outcomes by categories of antiretroviral therapy

Table E-1: Prevalence of metabolic abnormality outcomes by categories of antiretroviral therapy: exclusion of ART-naïve/ART status missing subjects

		Metabolic abnormality			Fasting hypertriglyceridemia			Hypercholesterolemia			Hypercholesterolemia and fasting hypertriglyceridemia		
		Prevalence	n/N	*p*-value	Prevalence	n/N	*p*-value	Prevalence	n/N	*p*-value	Prevalence	n/N	*p*-value
NRTI	Not current	100.00	1/1	0.101	100.00	1/1	0.032	50.00	1/2	0.130	50.00	1/2	0.005
	Current	26.97	96/356		17.60	63/359		13.30	48/361		5.03	18/358	
NNRTI	Not current	29.39	67/228	0.211	20.52	47/229	0.076	14.72	34/231	0.368	6.55	15/229	0.153
	Current	23.26	30/129		13.08	17/130		11.36	15/132		3.05	4/131	
PI	Not current	18.06	26.144	0.001	9.66	14/145	0.001	8.90	13/146	0.036	1.38	2/145	0.007
	Current	33.33	71/2130		23.36	50/214		16.59	36/217		7.91	17/215	

Association investigated using χ^2 test

E.2 Univariable analyses for metabolic abnormality

Table E-2: Univariable analyses for metabolic abnormality outcomes at recruitment

		Metabolic abnormality			Fasting hypertriglyceridemia			Hypercholesterolemia			Combined fasting hypertriglyceridemia, and hypercholesterolemia		
		OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Demographic factors													
Sex	**Male**	1			1			1			1		
	Female	0.82	(0.53, 1.28)	0.387	0.67	(0.40, 1.12)	0.130	0.93	(0.52, 1.65)	0.793	0.64	(0.27, 1.53)	0.312
Age (years)	**2-6**	1			1			1			1		
	7-11	0.97	(0.47, 2.01)	0.936	0.85	(0.38, 1.92)	0.695	0.93	(0.41, 2.07)	0.851	0.71	(0.25, 2.03)	0.525
	12-18	0.41	(0.20, 0.81)	0.011	0.44	(0.20, 0.95)	0.036	0.20	(0.09, 0.47)	<0.001	0.10	(0.03, 0.36)	<0.001
Ethnicity	**Black**	1			1			1			1		
	White	1.98	(1.12, 3.49)	0.019	2.49	(1.22, 5.09)	0.012	1.70	(0.82. 3.52)	0.155	2.27	(0.66, 7.88)	0.196
	Other	1.00	(0.26, 3.85)	1.000	1.27	(0.25, 6.36)	0.774	-	-	-	-	-	-
Country of residence	**Italy**	1			1			1			1		
	Belgium	0.71	(0.39, 1.32)	0.281	0.43	(0.20, 0.96)	0.038	1.09	(0.49, 2.43)	0. 826	0.93	(0.25, 3.41)	0.910
	Poland	1.46	(0.82, 2.61)	0.201	0.73	(0.35, 1.52)	0.398	3.96	(2.05, 7.65)	<0.001	3.29	(1.27, 8.53)	0.015
Infection factors													
CDC immune stage at recruitment	**Stage 1**	1			1			1			1		
	Stage 2	0.60	(0.33, 1.09)	0.093	0.95	(0.50, 1.81)	0.874	0.52	(0.23, 1.19)	0.122	1.14	(0.41, 3.19)	0.799
	Stage 3	0.36	(0.08, 1.61)	0.181	0.29	(0.04, 2.25)	0.238	0.36	(0.05, 2.75)	0.323	-	-	-

		Metabolic abnormality			Fasting hypertriglyceridemia			Hypercholesterolemia			Combined fasting hypertriglyceridemia, and hypercholesterolemia		
		OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Nadir CDC immune stage	**Stage 1**	1			1			1			1		
	Stage 2	0.68	(0.42, 1.12)	0.128	0.92	(0.52, 1.62)	0.773	0.49	(0.26, 0.94)	0.032	0.55	(0.21, 1.46)	0.229
	Stage 3	0.73	(0.40, 1.33)	0.304	0.73	(0.35, 1.52)	0.402	0.69	(0.32, 1.46)	0.327	0.67	(0.21, 2.17)	0.502
CDC clinical stage at recruitment	**N+A**	1			1			1			1		
	B	0.72	(0.23, 2.20)	0.559	0.85	(0.24, 2.98)	0.795	0.34	(0.04, 2.60)	0.300	-	-	-
	C	0.82	(0.17, 4.01)	0.805	0.60	(0.07, 4.88)	0.633	1.52	(0.32, 7.24)	0.603	-	-	-
Maximum CDC clinical stage	**N+A**	1			1			1			1		
	B	1.12	(0.68, 1.86)	0.655	0.95	(0.53, 1.72)	0.866	0.86	(0.44, 1.69)	0.665	0.43	(0.13, 1.37)	0.151
	C	0.91	(0.49, 1.67)	0.758	0.91	(0.46, 1.83)	0.799	1.02	(0.48, 2.17)	0.953	1.08	(0.39, 3.04)	0.878
Viral load (copies/ml)	**≤50**	1			1			1			1		
	>50	0.38	(0.21, 0.68)	0.001	0.64	(0.32, 1.26)	0.197	0.25	(0.13, 0.48)	<0.001	0.31	(0.12, 0.80)	0.015
Maximum duration of ART use	**Per year**	0.92	(0.87, 0.99)	0.017	0.97	(0.90, 1.04)	0.373	0.90	(0.83, 0.98)	0.017	0.98	(0.87, 1.11)	0.707
Other factors													
Hepatitis C co-infection	**Uninfected**	1			1			1			-		
	Infected	0.36	(0.11, 1.24)	0.106	0.40	(0.09, 1.75)	0.227	0.26	(0.03, 1.95)	0.189	-	-	-
Tanner score for puberty	**I**	1			1			1			1		
	II-IV	0.36	(0.21, 0.62)	<0.001	0.44	(0.24, 0.82)	0.009	0.21	(0.10, 0.45)	<0.001	0.15	(0.04, 0.52)	0.003
	V	0.38	(0.20, 0.70)	0.002	0.30	(0.14, 0.66)	0.003	0.27	(0.12, 0.60)	0.002	0.08	(0.01, 0.58)	0.013
BMI	**Kg/m2**	0.94	(0.88, 1.00)	0.052	0.95	(0.88, 1.02)	0.175	0.87	(0.79, 0.95)	0.002	0.86	(0.75, 0.99)	0.037

		Metabolic abnormality			Fasting hypertriglyceridemia			Hypercholesterolemia			Combined fasting hypertriglyceridemia, and hypercholesterolemia		
		OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value	OR	95% CI	*p*-value
NRTI													
Any NRTI	**Not current**	1			1			1			1		
	Current	1.05	(0.43, 2.57)	0.906	0.98	(0.36, 2.68)	0.972	0.77	(0.28, 2.10)	0.605	0.48	(0.13, 1.72)	0.258
NNRTI													
Any NNRTI	**Not current**	1			1			1			1		
	Current	0.75	(0.46, 1.23)	0.253	0.60	(0.33, 1.10)	0.098	0.75	(0.39, 1.41)	0.367	0.44	(0.15, 1.35)	0.152
PI													
Any PI	**Not current**	1			1			1			1		
	Current	2.16	(1.34, 3.48)	0.002	2.61	(1.46, 4.67)	0.001	1.84	(0.99, 3.40)	0.053	3.63	(1.20, 10.99)	0.023
Ritonavir booster	**Not current**	1			1			1			1		
	Current	2.67	(1.66, 4.30)	<0.001	3.08	(1.73, 5.46)	<0.001	2.36	(1.27, 4.36)	0.006	4.51	(1.49, 13.67)	0.008
Type of drug therapy at recruitment	**PI-based HAART**	1			1			1			1		
	NRTI immunotherapy	0.24	(0.07, 0.82)	0.023	0.25	(0.06, 1.09)	0.065	0.18	(0.02, 1.36)	0.096	-	-	-
	NNRTI-based HAART	0.52	(0.30, 0.89)	0.018	0.39	(0.20, 0.77)	0.007	0.57	(0.28, 1.15)	0.118	-	-	-
	Triple class	1.78	(0.58, 5.51)	0.317	1.50	(0.44, 5.09)	0.517	0.91	(0.19, 4.29)	0.904	0.20	(0.05, 0.90)	0.036

Odds ratio (OR) and accompanying 95% confidence interval (95% CI) for metabolic abnormality by participant characteristic. CDC-defined immune status - stage 1: no immunosuppression, stage 2: moderate immunosuppression, stage 3: severe immunosuppression. CDC-defined clinical status - N + A: no symptoms/asymptomatic, B: moderate symptoms, C: severe symptoms.

Table E-3: Univariable analyses for metabolic outcomes by ever-use of antiretroviral therapy

		Metabolic abnormality *n*= 415			Fasting hypertriglyceridemia *n*= 418			Hypercholesterolemia n=423			Combined hypercholesterolemia and Fasting hypertriglyceridemia n= 419		
		OR	(95% CI)	*p*	OR	(95% CI)	*p*	OR	(95% CI)	*p*	OR	(95% CI)	*p*
Ever use of ART	**No**	1			1			1			1		
	Yes	1.18	(0.46, 3.03)	0.724	1.19	(0.40, 3.55)	0.756	0.91	(0.30, 2.73)	0.866	0.70	(0.16, 3.16)	0.644
Any NRTI	**No**	1			1			1			1		
	Yes	1.18	(0.46, 3.03)	0.724	1.19	(0.40, 3.55)	0.756	0.91	(0.30, 2.73)	0.866	0.70	(0.16, 3.16)	0.644
Didanosine	**No**	1			1			1			1		
	Yes	1.25	(0.81, 1.95)	0.312	1.30	(0.78, 2.17)	0.312	0.78	(0.44, 1.38)	0.397	0.65	(0.27, 1.58)	0.340
Lamivudine	**No**	1			1			1			1		
	Yes	1.26	(0.60, 2.64)	0.539	1.41	(0.57, 3.47)	0.452	0.86	(0.36, 2.02)	0.723	0.79	(0.22, 2.78)	0.712
Stavudine	**No**	1			1			1			1		
	Yes	1.27	(0.82, 1.98)	0.284	1.44	(0.86, 2.42)	0.168	1.02	(0.58, 1.80)	0.938	0.95	(0.40, 2.23)	0.899
Zidovudine	**No**	1			1			1			1		
	Yes	1.03	(0.63, 1.68)	0.918	1.65	(0.88, 3.09)	0.119	0.63	(0.35, 1.14)	0.126	1.01	(0.38, 2.65)	0.985
Tenofovir	**No**	1			1			1			1		
	Yes	0.98	(0.61, 1.59)	0.947	1.17	(0.67, 2.02)	0.581	0.68	(0.35, 1.31)	0.250	0.88	(0.33, 2.29)	0.788
Any NNRTI	**No**	1			1			1			1		
	Yes	0.89	(0.57, 1.39)	0.619	0.83	(0.49, 1.38)	0.467	0.59	(0.34, 1.04)	0.069	0.32	(0.13, 0.81)	0.016
Efavirenz	**No**	1			1			1			1		
	Yes	0.86	(0.54, 1.35)	0.510	0.83	(0.49, 1.42)	0.502	0.57	(0.31, 1.07)	0.082	0.35	(0.12, 1.06)	0.062
Nevirapine	**No**	1			1			1			1		
	Yes	1.07	(0.64, 1.78)	0.799	1.06	(0.59, 1.92)	0.838	0.71	(0.35, 1.43)	0.337	0.47	(0.14, 1.62)	0.231
Any PI	**No**	1			1			1			1		
	Yes	1.93	(1.07, 3.47)	0.030	1.55	(0.79, 3.01)	0.201	2.24	(0.98, 5.12)	0.056	1.91	(0.55, 6.60)	0.306
Ritonavir booster	**No**	1			1			1			1		
	Yes	2.08	(1.29, 3.37)	0.003	1.96	(1.11, 3.46)	0.020	2.14	(1.13, 4.07)	0.019	3.10	(1.03, 9.34)	0.044
Indinavir	**No**	1			1			1			1		

		Metabolic abnormality			Fasting hypertriglyceridemia			Hypercholesterolemia			Combined hypercholesterolemia and Fasting hypertriglyceridemia		
		n= 415			*n*= 418			n=423			n= 419		
		OR	**(95% CI)**	***p***	**OR**	**(95% CI)**	***p***	**OR**	**(95% CI)**	***p***	**OR**	**(95% CI)**	***p***
	Yes	0.84	(0.33, 2.16)	0.724	1.18	(0.43, 3.23)	0.753	0.25	(0.03, 1.87)	0.177	-	-	-
Nelfinavir	**No**	1			1			1			1		
	Yes	1.40	(0.90, 2.17)	0.135	1.20	(0.72, 2.00)	0.484	1.19	(0.68, 2.09)	0.542	0.90	(0.38, 2.13)	0.808
Saquinavir	**No**	1			1			1			1		
	Yes	1.86	(0.70, 4.94)	0.210	2.37	(0.87, 6.47)	0.092	0.35	(0.05, 2.69)	0.315	-	-	-

E.3 Multivariable analyses for metabolic abnormality

Table E-4: Final multivariable models for metabolic outcomes with ever-use of specific antiretroviral drugs as explanatory variables

		Any metabolic abnormality			Any fasting hypertriglyceridemia			Any hypercholesterolemia			Combined hypercholesterolemia, and fasting hypertriglyceridemia		
		n	*n* = 341 AOR (95% CI)	*p*-value	*n*	*n* = 356 AOR (95% CI)	*p*-value	*n*	*n* = 358 AOR (95% CI)	*p*-value	*n*	*n* = 356 AOR (95% CI)	*p*-value
Age	**Per year**	341	0.87 (0.81, 0.94)	<0.001	356	0.89 (0.82, 0.97)	0.008	358	0.82 (0.75, 0.90)	<0.001	356	0.71 (0.60, 0.84)	<0.001
ART duration	**Per Year**	341	0.96 (0.89, 1.04)	0.298	356	1.00 (0.92, 1.09)	0.962	358	0.99 (0.90, 1.10)	0.891	356	1.20 (1.00, 1.43)	0.049
Sex	**Male**												
	Female												
Ethnicity	**Black**	88	1										
	White	236	2.08 (1.01, 4.29)	0.048									
	Other	17	0.86 (0.21, 3.59)	0.836									
Viral load (HIV-RNA)	**<50 copies/ml**							128	1				
	≥50 copies/ml							230	2.77 (1.26, 6.10)	0.011			
Ritonavir booster	**Never**	122	1		127	1		128	1		128	1	
	Ever	219	2.11 (1.15, 3.87)	0.016	229	2.08 (1.04, 4.18)	0.038	230	2.77 (1.26, 6.10)	0.011	228	5.49 (1.22, 24.62)	0.026

Models constructed using backwards stepwise logistic regression.

Table E-5: Sensitivity analyses: Multivariable models for moderate/severe metabolic abnormality outcomes

		Any metabolic abnormality			Any fasting hypertriglyceridemia			Any hypercholesterolemia			Combined hypercholesterolemia, and fasting hypertriglyceridemia		
		n	*n* = 315 AOR (95% CI)	*p*-value	*n*	*n* = 309 AOR (95% CI)	*p*-value	*n*	*n* = 321 AOR (95% CI)	*p*-value	*n*	*n* = 321 AOR (95% CI)	*p*-value
Age	**Per year**	315	0.90 (0.82, 0.98)	0.019	309	0.95 (0.86, 1.05)	0.362	321	0.76 (0.64, 0.90)	0.001	321	0.76 (0.63, 0.91)	0.003
ART duration	**Per Year**	315	1.01 (0.91, 1.11)	0.866	309	1.00 (0.90, 1.11)	0.943	321	1.11 (0.93, 1.34)	0.248	321	1.07 (0.88, 1.32)	0.491
Sex	**Male**				161	1							
	Female				148	0.44 (0.20, 0.96)	0.040						
Ethnicity	**Black**	77	1		79	1							
	White	223	2.12 (0.77, 5.87)	0.148	215	2.53 (0.80, 8.05)	0.115						
	Other	15	0.69 (0.07, 6.62)	0.748	15	1.01 (0.10, 10.32)	0.992						
Viral load (HIV-RNA)	**<50 copies/ml**				195	1		203	1				
	≥50 copies/ml				114	1.18 (0.54, 2.60)	0.679	118	0.80 (0.24, 2.63)	0.716			
Ritonavir booster	**Not current**	144	1		141	1		147	1		147	1	
	Current	171	4.65 (1.97, 10.99)	<0.001	168	4.35 (1.72, 11.03)	0.002	174	4.04 (1.09, 14.96)	0.037	174	4.68 (0.98, 22.42)	0.054

Models constructed using backwards stepwise logistic regression.

Table E-6: Final multivariable models for metabolic outcomes adjusted by Tanner score for puberty

		Any metabolic abnormality			Any fasting hypertriglyceridemia			Any hypercholesterolemia			Combined hypercholesterolemia, and fasting hypertriglyceridemia		
		n	*n* = 278 AOR (95% CI)	*p*-value	*n*	*n* = 272 AOR (95% CI)	*p*-value	*n*	*n* = 293 AOR (95% CI)	*p*-value	*n*	*n* = 292 AOR (95% CI)	*p*-value
Age	**Per year**	278	0.98 (0.85, 1.11)	0.712	272	0.96 (0.82, 1.13)	0.623	293	0.86 (0.74, 1.01)	0.062	292	0.73 (0.55, 0.95)	0.021
ART duration	**Per Year**	278	0.95 (0.87, 1.04)	0.278	272	0.97 (0.87, 1.08)	0.564	293	1.02 (0.91, 1.14)	0.777	292	1.28 (1.04, 1.59)	0.022
Tanner score puberty	**I**	101	1		98	1		107	1		107	1	
	II-IV	111	0.37 (0.15, 0.94)	0.036	106	0.40 (0.13, 1.24)	0.113	115	0.47 (0.15, 1.46)	0.190	116	0.34 (0.05, 2.43)	0.282
	V	66	0.32 (0.09, 1.22)	0.095	68	0.30 (0.06, 1.50)	0.143	71	0.89 (0.19, 4.17)	0.884	69	0.71 (0.04, 12.65)	0.817
Sex	**Male**				136	1							
	Female				136	0.80 (0.39, 1.60)	0.522						
Ethnicity	**Black**	69	1		70	1							
	White	194	2.00 (0.90, 4.47)	0.090	187	2.46 (0.83, 7.27)	0.103						
	Other	15	0.85 (0.19, 3.80)	0.827	15	0.92 (0.14, 6.23)	0.936						
Viral load (HIV-RNA)	**<50 copies/ml**				169	1		181	1				
	≥50 copies/ml				103	1.51 (0.69, 3.30)	0.304	112	0.41 (0.18, 0.93)	0.033			
Ritonavir booster	**Not current**	124	1		121	1		132	1		131	1	
	Current	154	2.25 (1.22, 4.16)	0.009	151	2.87 (1.29, 6.39)	0.010	161	2.65 (1.24, 5.67)	0.012	161	13.19 (1.69, 103.1)	0.014

Original models constructed using backward stepwise logistic regression: additional adjustment with Tanner score for puberty

Table E-7: Final multivariable models for fat disorder outcome with the addition of an interaction between sex and age

		Any metabolic abnormality			Any fasting hypertriglyceridemia			Any hypercholesterolemia			Combined hypercholesterolemia, and fasting hypertriglyceridemia		
		n	*n* = 308 AOR (95% CI)	*p*-value	*n*	*n* = 309 AOR (95% CI)	*p*-value	*n*	*n* = 324 AOR (95% CI)	*p*-value	*n*	*n* = 322 AOR (95% CI)	*p*-value
Age	**Per year**	308	0.91 (0.82, 1.00)	0.047	309	0.93 (0.83, 1.04)	0.192	324	0.76 (0.66, 0.87)	<0.001	322	0.65 (0.52, 0.82)	<0.001
ART duration	**Per Year**	308	0.97 (0.089, 1.05)	0.447	309	0.98 (0.89, 1.08)	0.740	324	1.02 (0.91, 1.14)	0.752	322	1.25 (1.02, 1.52)	0.028
Sex	**Male**	161	1		161	1		167	1		167	1	
	Female	147	2.39 (0.41,13.96)	0.333	148	3.74 (0.50, 27.46)	0.199	157	0.22 (0.03, 1.55)	0.129	155	0.16 (0.01, 2.35)	0.182
Age*Female Interaction		147	0.93 (0.08, 1.08)	0.319	309	0.93 (0.83, 1.04)	0.192	157	1.18 (0.98, 1.41)	0.083	155	1.18 (0.90, 1.55)	0.221
Ethnicity	**Black**	76	1		79	1							
	White	217	1.61 (0.74, 3.48)	0.230	215	2.33 (0.85, 6.34)	0.099						
	Other	15	0.597 (0.15, 2.98)	0.597	15	0.81 (0.13, 5.07)	0.820						
Viral load (HIV-RNA)	**<50 copies/ml**				195	1		204	1				
	≥50 copies/ml				114	1.30 (0.63, 2.70)	0.476	120	0.35 (0.15, 0.81)	0.015			
Ritonavir booster	**Not current**	140	1		141	1		148	1		147	1	
	Current	168	2.91 (1.59, 5.34)	0.001	168	3.69 (1.73, 7.87)	0.001	176	2.76 (1.29, 5.89)	0.009	175	7.18 (1.58, 32.62)	0.011

Original models constructed using backward stepwise logistic regression: additional adjustment with interaction between sex and age

Table E-8: Multivariable logistic regressions for metabolic abnormality outcomes including categories of ART as explanatory variables: threshold for stepwise covariate selection set at 10% significance

		Metabolic abnormality	Fasting hypertriglyceridemia	Hypercholesterolemia	Both hypercholesterolemia and fasting hyoertriglyceridemia
		n = 326	*n* = 317	*n* = 331	*n* =328
Age	**Per year**	0.89 (0.82, 0.95)***	0.88 (0.80, 0.96)**	0.82 (0.75, 0.90)***	0.71 (0.59, 0.85)***
Ethnicity	**Black**		1		
	White		2.44 (0.92, 6.49)†		
	Other		0.94 (0.15, 5.75)		
Detectable viral load	**≤50 copies/ml**			1	1
	>50 copies/ml			0.40 (0.18, 0.89)*	0.33 (0.09, 1.23)†
Duration of ART	**Per year**	0.97 (0.90, 1.05)	0.99 (0.91, 1.09)	1.00 (0.90, 1.11)	1.24 (1.02, 1.50)*
NNRTI at recruitment	**No**	1			
	Yes	2.11 (0.87, 5.11)†			
PI at recruitment	**No**	1	1	1	1
	Yes	4.17 (1.72, 10.15)**	2.79 (1.35, 5.77)**	2.21 (1.07, 4.56)*	6.31 (1.39, 28.68)*

†0.10 > p ≥ 0.05, *0.05 > p ≥> 0.01, **0.01 > p ≥ 0.001, ***0.001< p

Table E-9: Multivariable logistic regressions for metabolic abnormality outcomes including specific ART drugs as explanatory variables: threshold for stepwise covariate selection set at 10% significance

		Metabolic abnormality *n* = 315	Fasting hypertriglyceridemia *n* = 328	Hypercholesterolemia *n* = 331	Both hypercholesterolemia and fasting hypertriglyceridemia *n* = 328
Age	**Per year**	0.87 (0.81, 0.94)***	0.88 (0.81, 0.97)**	0.82 (0.75, 0.90)***	0.71 (0.60, 0.86)***
Ethnicity	**Black**	1			
	White	1.64 (0.77, 3.49)			
	Other	0.75 (0.17, 3.25)			
Detectable viral load	**≤50 copies/ml**			1	1
	>50 copies/ml			0.39 (0.17, 0.86)*	0.30 (0.08, 1.12)†
Duration of ART	**Per Year**	0.97 (0.90, 1.05)	1.01 (0.92, 1.11)	1.01 (0.91, 1.12)	1.25 (1.03, 1.53)*
Ritonavir-booster at recruitment	**No**	1	1	1	1
	Yes	2.79 (1.56, 4.99)***	6.17 (2.32, 16.40)***	2.82 (1.36, 5.83)**	8.06 (1.76, 36.81)**
Efavirenz at recruitment	**No**		1		
	Yes		2.57 (0.88, 7.47)†		

†0.10 > p ≥ 0.05, *0.05 > p ≥> 0.01, **0.01 > p ≥ 0.001, ***0.001< p

E.4 Prevalence of lipodystrophy syndrome by categories of antiretroviral therapy

Table E-10: Prevalence of lipodystrophy syndrome by categories of antiretroviral therapy: exclusion of ART-naïve/ART status missing subjects

		Prevalence	*n/N*	*p*-value
NRTI	**Not current**	100.00	2/2	0.245
	Current	59.55	212/356	
NNRTI	**Not current**	58.95	135/229	0.672
	Current	61.24	79/129	
PI	**Not current**	54.55	78/143	0.100
	Current	63.26	136/215	

Association investigated using χ^2 test

E.5 Univariable analyses for lipodystrophy syndrome

Table E-11: Univariable analyses for lipodystrophy syndrome by ever-use of antiretroviral therapy

		Lipodystrophy syndrome *n*= 416		
		OR	**(95% CI)**	***p***
Ever use of ART	**No**	1		
	Yes	2.60	(1.13, 5.98)	0.024
Any NRTI	**No**	1		
	Yes	2.60	(1.13, 5.98)	0.024
Didanosine	**No**	1		
	Yes	2.46	(1.65, 3.67)	<0.001
Lamivudine	**No**	1		
	Yes	1.72	(0.92, 3.21)	0.087
Stavudine	**No**	1		
	Yes	2.95	(1.97, 4.41)	<0.001
Zidovudine	**No**	1		
	Yes	1.27	(0.82, 1.96)	0.283
Tenofovir	**No**	1		
	Yes	1.32	(0.86, 2.03)	0.199
Any NNRTI	**No**	1		
	Yes	1.43	(0.97, 2.12)	0.073
Efavirenz	**No**	1		
	Yes	1.61	(1.07, 2.42)	0.021
Nevirapine	**No**	1		
	Yes	1.08	(0.69, 1.71)	0.727
Any PI	**No**	1		
	Yes	2.20	(1.38, 3.53)	0.001
Ritonavir booster	**No**	1		
	Yes	2.01	(1.35, 3.01)	0.001
Indinavir	**No**	1		
	Yes	4.57	(1.55, 13.51)	0.006
Nelfinavir	**No**	1		
	Yes	1.84	(1.24, 2.73)	0.002
Saquinavir	**No**	1		
	Yes	6.54	(1.48, 28.82)	0.013

E.6 Multivariable analyses for lipodystrophy syndrome

Table E-12: Final multivariable model for lipodystrophy syndrome with ever-use of specific antiretroviral drugs as explanatory variables (*n* = 338)

		n	AOR (95% CI)	*p* – value
Age	**Per year**	338	0.90 (0.83, 0.98)	0.018
Duration of ART use	**Per year**	338	0.93 (0.87, 1.00)	0.052
Ethnicity	**Black**	90	1	
	White	231	3.09 (1.77, 5.39)	<0.001
	Other	17	0.72 (0.23, 2.27)	0.573
BMI	**Per kg/m^2**	338	1.11 (1.02, 1.21)	0.021
Abacavir	**Never use**	188	1	
	Ever use	150	0.57 (0.35, 0.93)	0.023
Stavudine	**Never use**	153	1	
	Ever use	185	2.24 (1.29, 3.88)	0.004
Didanosine	**Never use**	167	1	
	Ever use	171	1.95 (1.09, 3.49)	0.024
Indinavir	**Never use**	318	1	
	Ever use	20	4.20 (1.08, 16.37)	0.038

Models constructed using backward stepwise logistic regression.

Table E-13: Final multivariable models for lipodystrophy syndrome adjusted by Tanner score for puberty (*n* = 279)

		n	AOR (95% CI)	*p* – value
Age	**Per year**	279	0.94 (0.83, 1.07)	0.333
Duration of ART use	**Per year**	279	1.04 (0.96, 1.12)	0.385
Tanner score for puberty	**I**	101	1	
	II-IV	112	0.75 (0.31, 1.80)	0.521
	V	66	1.10 (0.32, 3.75)	0.881
Ethnicity	**Black**	70	1	
	White	194	3.54 (1.85, 6.77)	<0.001
	Other	15	0.70 (0.20, 2.49)	0.579
Body mass index (BMI)	**Per m/kg^2**	279	1.10 (1.00, 1.21)	0.046
Efavirenz	**Not current**	209	1	
	Current	70	4.64 (1.94, 11.11)	0.001
Nevirapine	**Not current**	248	1	
	Current	31	3.80 (1.31, 11.0)	0.014
Ritonavir booster	**Not current**	124	1	
	Current	155	4.03 (1.84, 8.85)	0.001

Original model constructed using backward stepwise logistic regression: additional adjustment with Tanner score for puberty

Table E-14: Final multivariable models for lipodystrophy syndrome with the addition of an interaction between sex and age (*n* = 306)

		n	AOR (95% CI)	*p* – value
Age	**Per year**	306	0.96 (0.87, 1.05)	0.363
Duration of ART use	**Per year**	306	1.02 (0.95, 1.10)	0.648
Gender	**Male**	159	1	
	Female	147	1.84 (0.35, 9.74)	0.474
Gender * Age interaction		147	0.97 (0.85, 1.11)	0.648
Ethnicity	**Black**	78	1	
	White	213	3.42 (1.87, 6.26)	<0.001
	Other	15	0.70 (0.20, 2.47)	0.579
Body mass index	**Per m/kg^2**	306	1.11 (1.01, 1.21)	0.025
Efavirenz	**Not current**	231	1	
	Current	75	2.79 (1.29, 6.07)	0.009
Nevirapine	**Not current**	274	1	
	Current	32	2.95 (1.11, 7.86)	0.030
Ritonavir booster	**Not current**	139	1	
	Current	167	3.08 (1.54, 6.15)	0.001

Original model constructed using backward stepwise logistic regression: additional adjustment with interaction between sex and age

Table E-15: Multivariable logistic regressions for lipodystrophy syndrome including categories of ART as explanatory variables: threshold for stepwise covariate selection set at 10% significance

		Lipodystrophy syndrome *n* = 298
Age	**Per year**	0.93 (0.86, 1.01)†
Ethnicity	**Black**	1
	White	3.24 (1.78, 5.88)***
	Other	0.90 (0.26, 3.19)
Body mass index	**Kg/m^2**	1.09 (1.00, 1.19)†
Maximum CDC-defined	**N/A**	1
clinical condition	**B**	1.89 (1.07, 3.36)*
	C	1.65 (0.86, 3.16)
Duration of ART	**Per year**	1.01 (0.94, 1.09)
PI at recruitment	**No**	1
	Yes	2.40 (1.14, 5.05)*
NNRTI at recruitment	**No**	1
	Yes	2.76 (1.27, 6.00)*

†**0.10 > p ≥ 0.05, *0.05 > p ≥> 0.01, **0.01 > p ≥ 0.001, ***0.001< p**

Table E-16: Multivariable logistic regressions for lipodystrophy syndrome including categories of ART as explanatory variables: threshold for stepwise covariate selection set at 10% significance

		Lipodystrophy syndrome *n* = 298
Age	**Per year**	0.93 (0.86, 1.02)
Ethnicity	**Black**	1
	White	2.72 (1.50, 4.92)***
	Other	0.88 (0.25, 3.13)
Body mass index	**Kg/m^2**	1.10 (1.01, 1.20)*
Maximum CDC-defined clinical status	**N/A**	1
	B	1.79 (1.00, 3.19)*
	C	1.56 (0.82, 2.98)
Duration of ART use	**Per year**	1.02 (0.95, 1.10)
Stavudine at recruitment	**No**	1
	Yes	2.48 (1.01, 6.08)*
Efavirenz at recruitment	**No**	1
	Yes	2.27 (1.11, 4.65)*
Ritonavir booster at recruitment	**No**	1
	Yes	2.14 (1.14, 3.99)*

†0.10 > p ≥ 0.05, *0.05 > p ≥> 0.01, **0.01 > p ≥ 0.001, ***0.001< p

Appendix F Appendix to chapter 6

F.1 Classification of body mass index

Table F-1: International classification of adult underweight, overweight and obesity according to body mass index

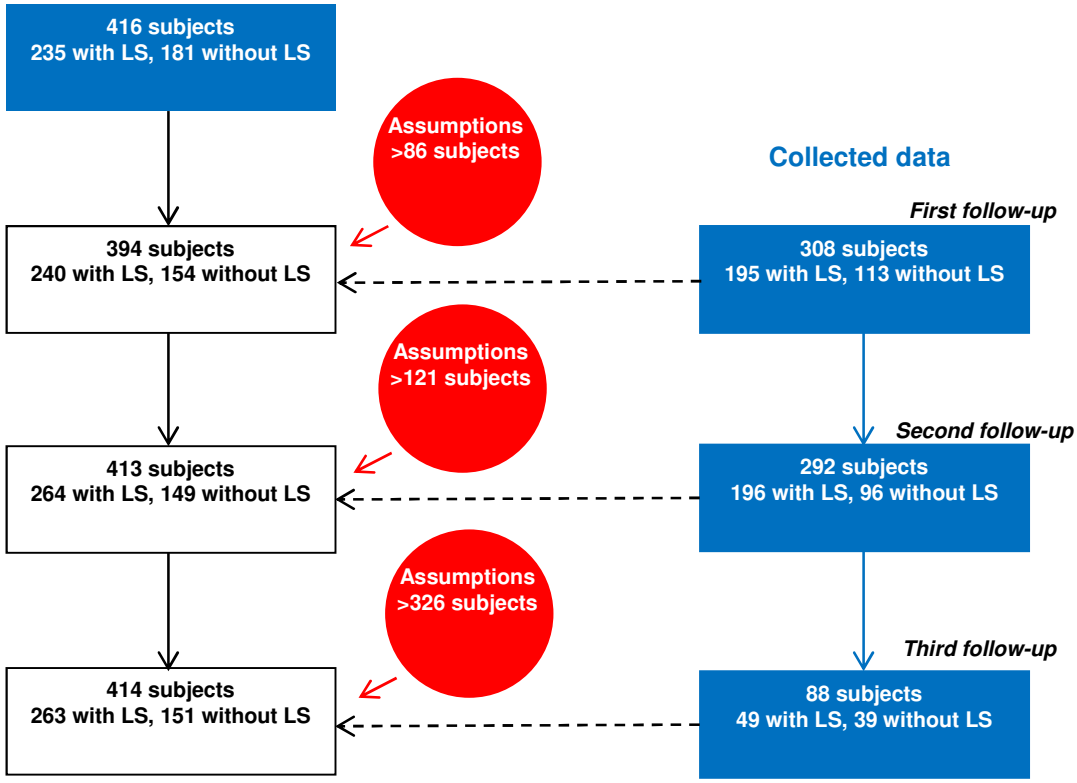
	BMI range (kg/m^2)
Severely underweight	<16.00
Underweight	16.0 – 18.5
Healthy weight	18.5 – 25.0
Overweight	25.0 – 30.0
Obese	>30.0

F.2 Assumptions of LS status over follow-up

Specific data was collected for 416 subjects regarding LS status at recruitment. Following this, 308 subjects had data collected in a second questionnaire, 292 in a third questionnaire, and 88 subjects in a fourth questionnaire. Despite, the diminishing number of subjects at each follow-up point, specific assumptions could be made regarding a proportion of subjects at each follow-up. Figure F-1 illustrates the number of questionnaires collected with specific LS data during active surveillance of the cohort. The number of subjects where assumptions could be made at each time point is also illustrated. Assumptions were made regarding the presence or absence of LS symptoms dependent on data collected at the previous time point: where this was not possible, no assumptions were made and LS status was classed as “missing”. The minimum number of subjects where assumptions were possible is illustrated as LS status does not remain constant in a given subject over time (i.e. new cases can occur and complete reversal of symptoms can occur).

Similar assumptions regarding other LS symptoms (e.g. specific body fat alterations and metabolic abnormality) with continuing surveillance were made.

Figure F-1: Assumptions regarding lipodystrophy syndrome symptoms with follow-up



These assumptions were investigated by calculating the prevalence of LS as estimated from the first, second and third follow-up study visit questionnaires collected for each variable.

Table F-2: Prevalence of lipodystrophy outcomes as estimated from follow-up questionnaires

	First follow-up		Second follow-up		Third follow-up		Data at the end of follow-up (with assumptions)	
	n = 308		*n* = 292		*n* = 88		*n* = 414	
	n/*N*	%	*n*/*N*	%	*n*/*N*	%	*n*/*N*	%
Lipodystrophy syndrome	195/308	63.3	196/292	67.1	49/88	55.7	253/414	61.1
Body fat alterations only	91/308	29.5	110/292	37.7	20/88	22.7	120/414	29.0
Metabolic abnormality only	54/308	17.5	39/292	13.4	20/88	22.7	72/414	17.4
Both body fat alterations and metabolic abnormality	50/308	16.2	47/292	16.1	9/88	10.2	61/414	14.7
Lipoatrophy only	53/308	17.2	56/292	19.2	12/88	13.6	63/414	15.2
Lipohypertrophy only	39/308	12.7	56/292	19.2	10/88	11.4	54/414	13.0
Both lipoatrophy and lipohypertrophy	53/308	17.2	56/292	19.2	12/88	13.6	64/414	15.5
Fasting hypertriglyceridemia only	34/208	11.0	26/292	8.9	1/88	1.1	39/414	9.4
Hypercholesterolemia only	58/208	18.8	51/292	17.5	26/88	29.5	60/414	14.5
Both hypercholesterolemia and fasting hypertriglyceridemia	12/308	3.9	9/292	3.1	2/88	2.3	15/414	3.6

F.3 Hospital visits

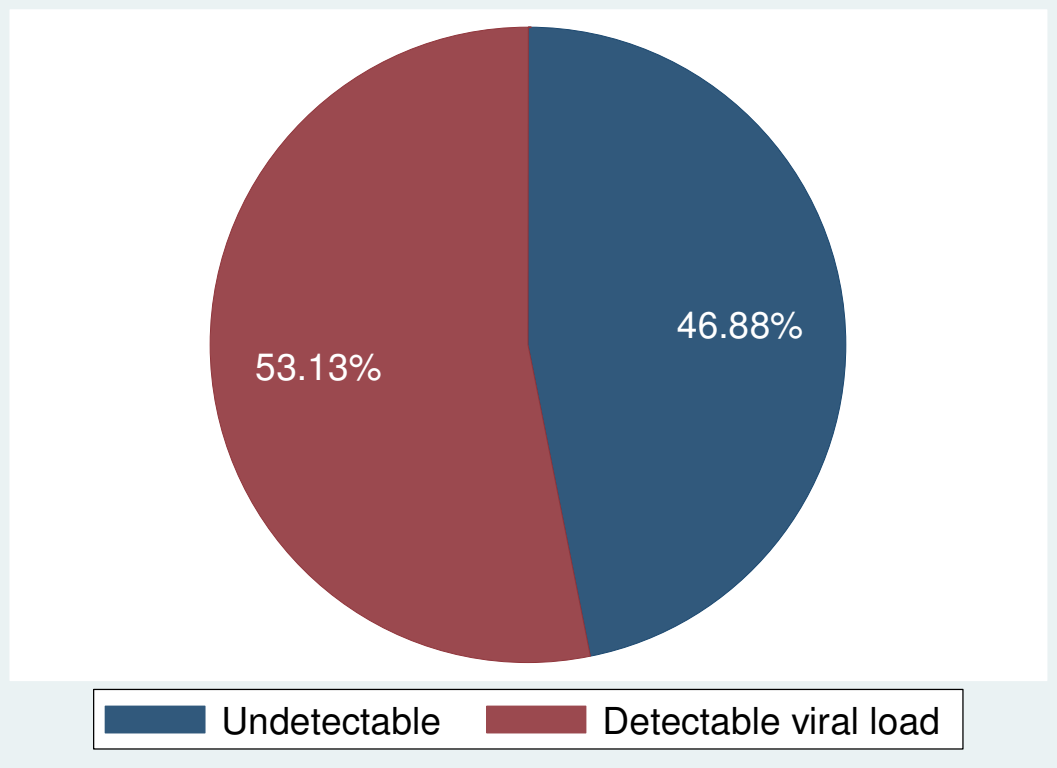
Table F-3: Median interval between hospital visits (years)

	Hospital visit		
	1st and 2nd visit*	2nd and 3rd visit*	3rd and 4th visit*
Belgium	0.54 (0.46, 0.61)	0.63 (0.48, 0.92)	0.61 (0.50, 0.99)
Italy	1.21 (1.04, 1.50)	2.10 (1.87, 2.30)	0
Poland	0.50 (0.48, 0.52)	0.51 (0.49, 0.53)	0.51 (0.50, 0.53)
TOTAL	1.00 (0.54, 1.28)	1.71 (0.56, 2.16)	0.53 (0.50, 0.68)

No 4th visit data was collected for subjects resident in Italy. * Mann-Whitney-U test for medians between countries: $p < 0.001$

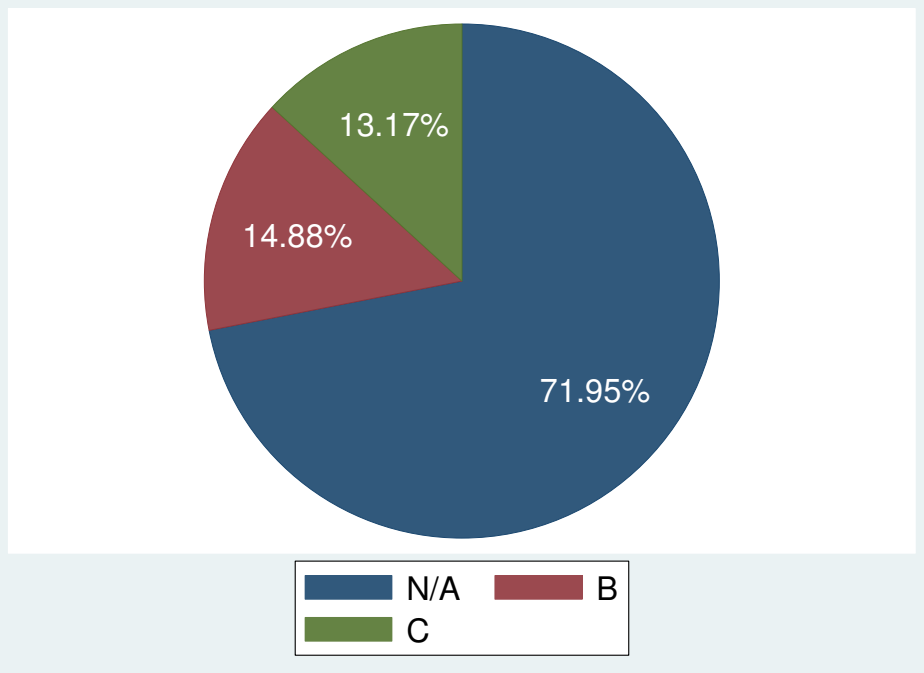
F.3 Characteristics of participants at end of follow-up

Figure F-2: Prevalence of detectable viral load at the end of follow-up (n = 416)



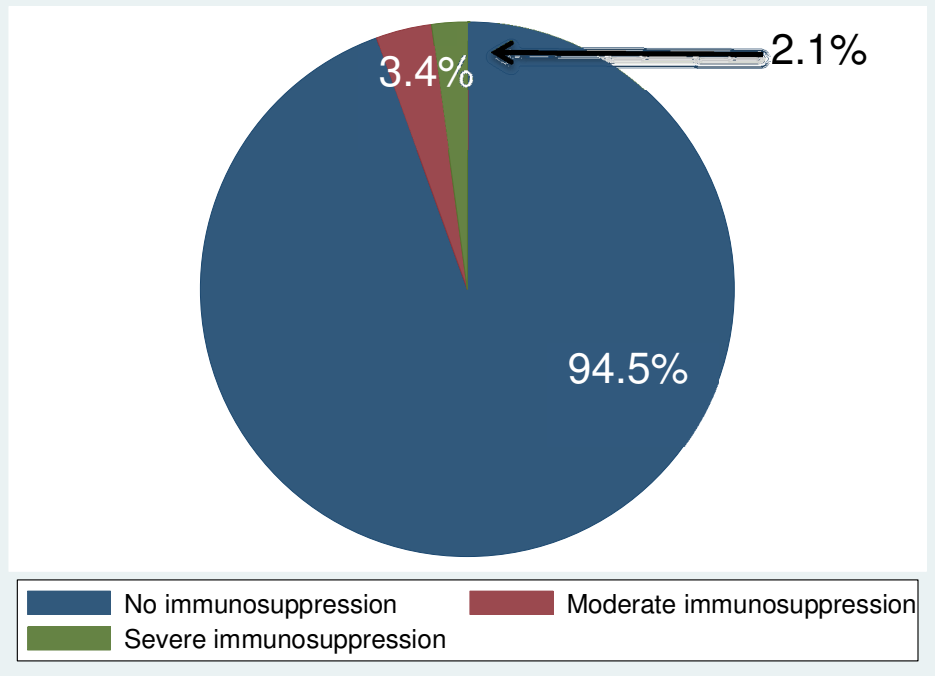
Detectable viral load defined as >50 copies HIV-RNA per mL.

Figure F-3: Prevalence of CDC-defined clinical condition at the end of follow-up (*n* = 410)



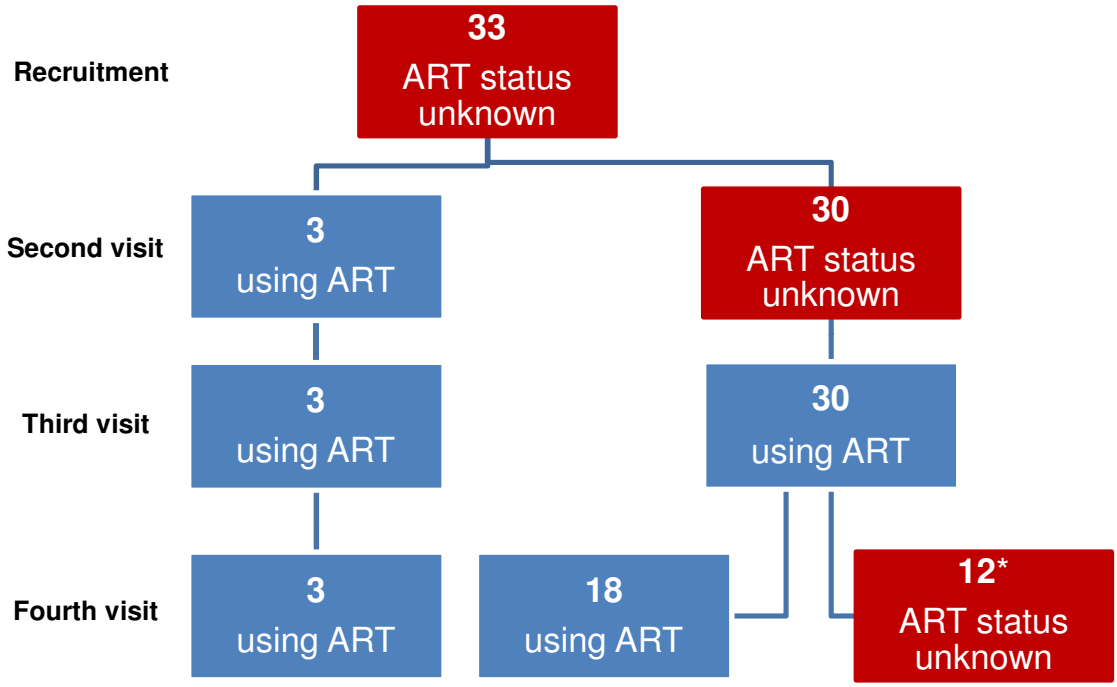
CDC-defined clinical status - N + A: no symptoms/asymptomatic, B: moderate symptoms, C: severe symptoms

Figure F-4: Prevalence of CDC-defined immunosuppression at the end of follow-up (*n* = 416)



F.4 Follow-up of subjects who were ART-naïve at recruitment

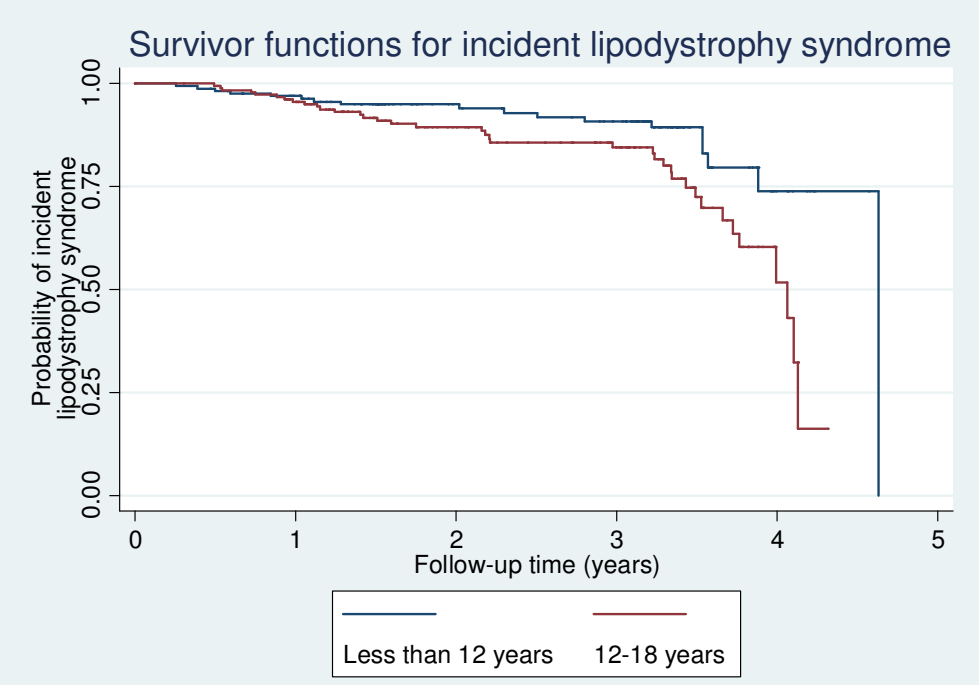
Figure F-5: Flow chart of subjects whose antiretroviral therapy status was unknown at recruitment

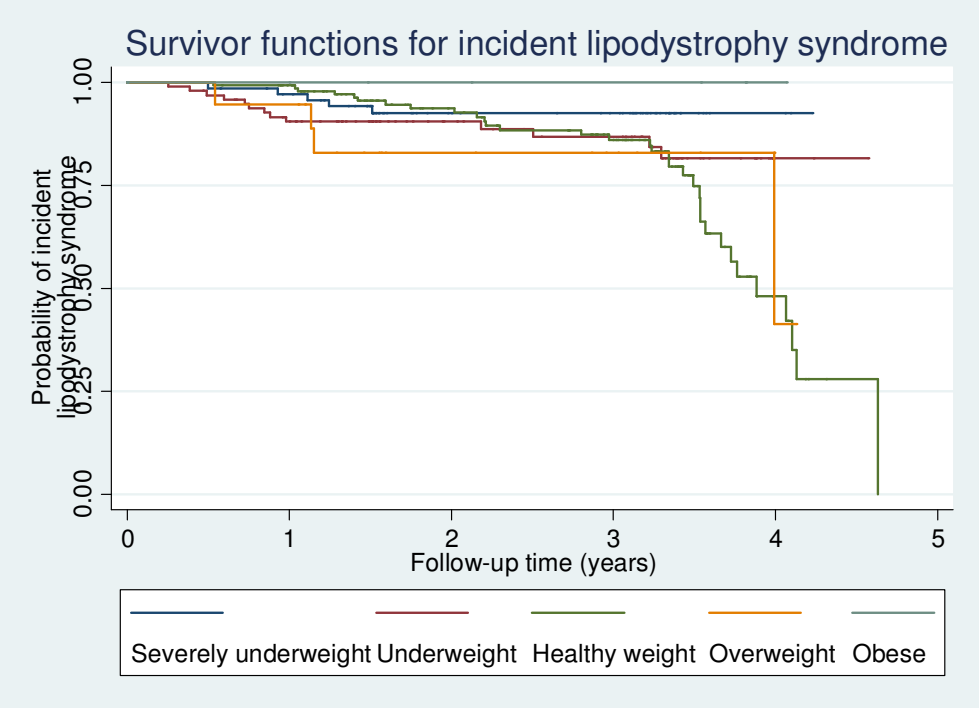


*Italian cases: no fourth visit

F.5 Kaplan-Meier survival plots

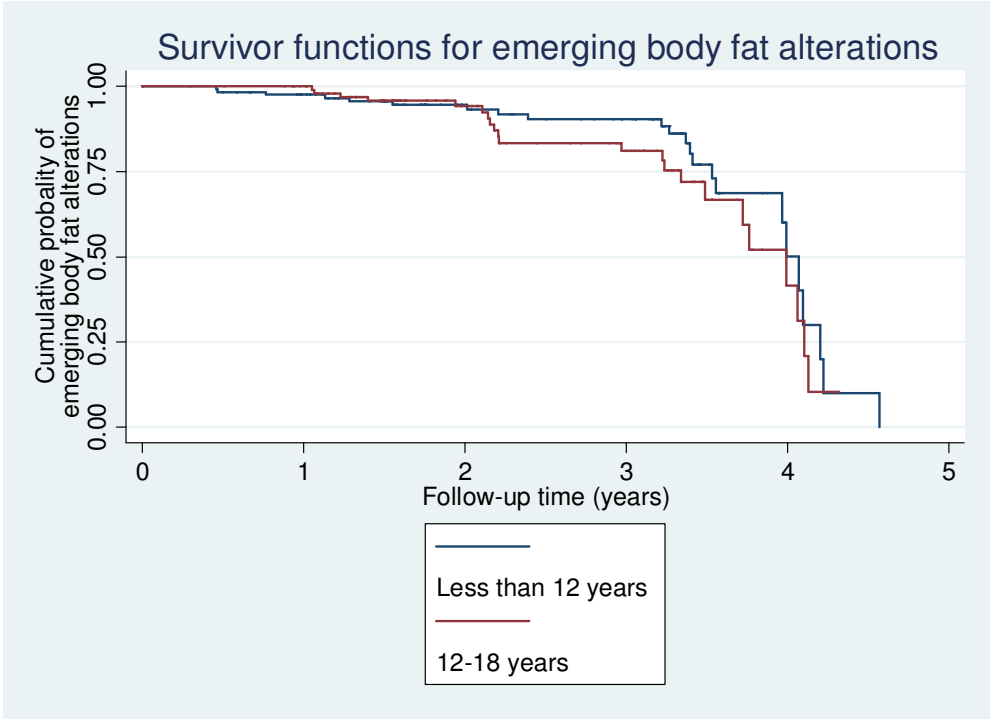
Figure F-6: Kaplan-Meir estimates for incidence lipodystrophy syndrome by (a) age at recruitment, and (b) and BMI at recruitment

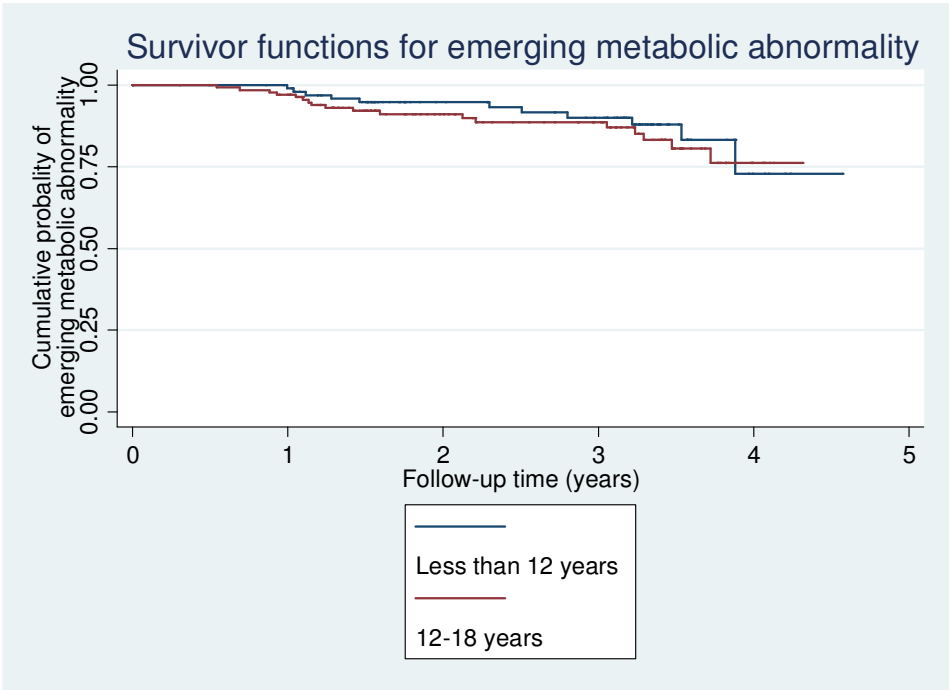




Excludes 205 subjects with continuous lipodystrophy syndrome over follow-up and17 subjects with >1 occasion of incidence/complete reversal of LS over follow-up. Log rank test: (a) $p = 0.036$, and (b) $p = 0.476$.

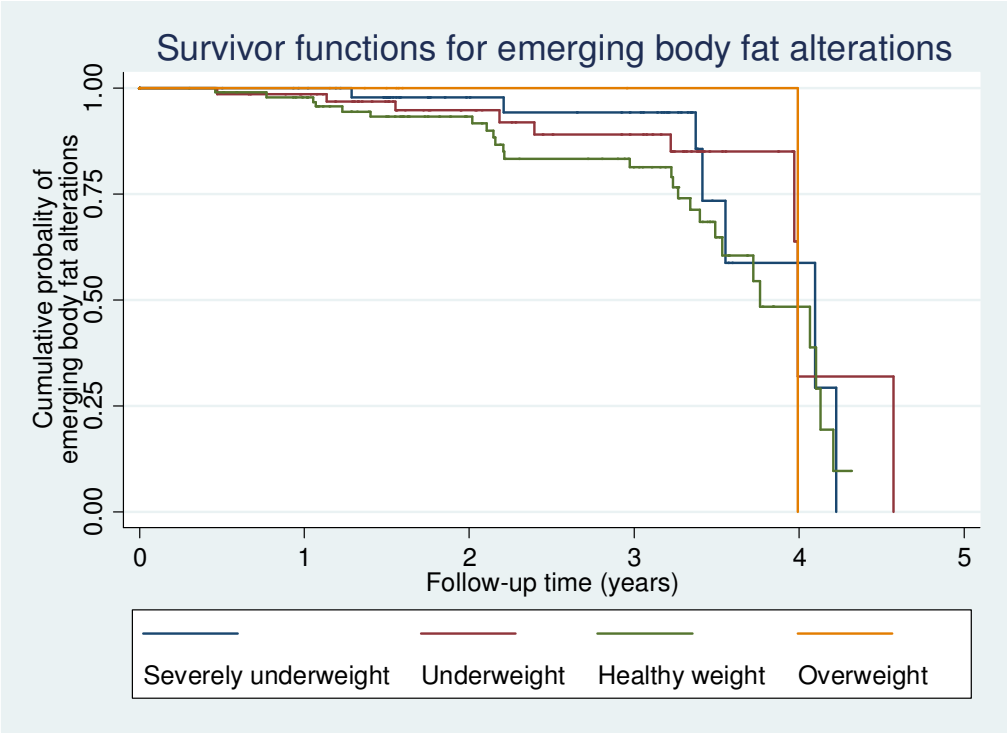
Figure F-7: Kaplan-Meier plots for emergence of (a) body fat alterations and (b) metabolic abnormality, by age at recruitment

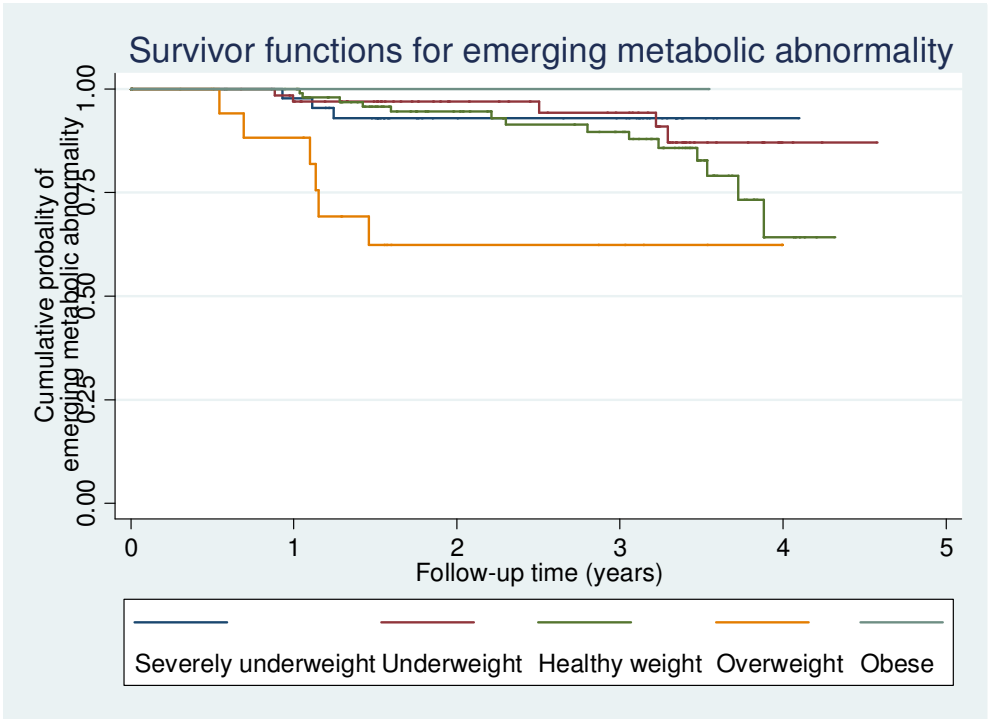




Estimates exclude 122 subjects with continuous fat alterations over follow-up and 19 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up (a), and 106 subjects with continuous metabolic abnormality over follow-up and 20 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up, (b). Log-rank test: (a) 0.069, and (b) 0.581.

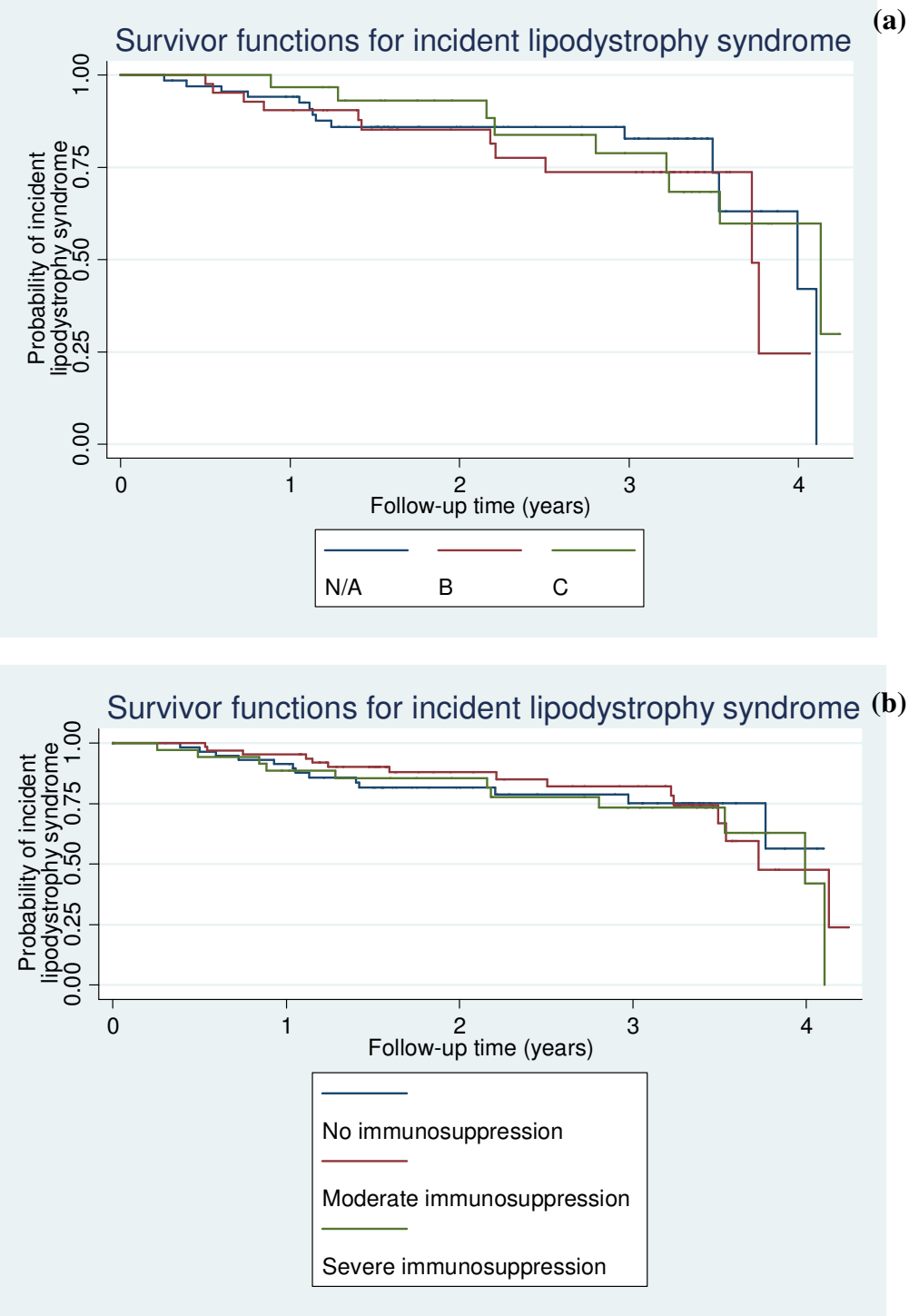
Figure F-8: Kaplan-Meier plots for emergence of (a) body fat alterations and (b) metabolic abnormality, by BMI at recruitment





Estimates exclude 122 subjects with continuous fat alterations over follow-up and 19 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up (a), and 106 subjects with continuous metabolic abnormality over follow-up and 20 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up, (b). Log-rank test: (a) 0.179, and (b) 0.099.

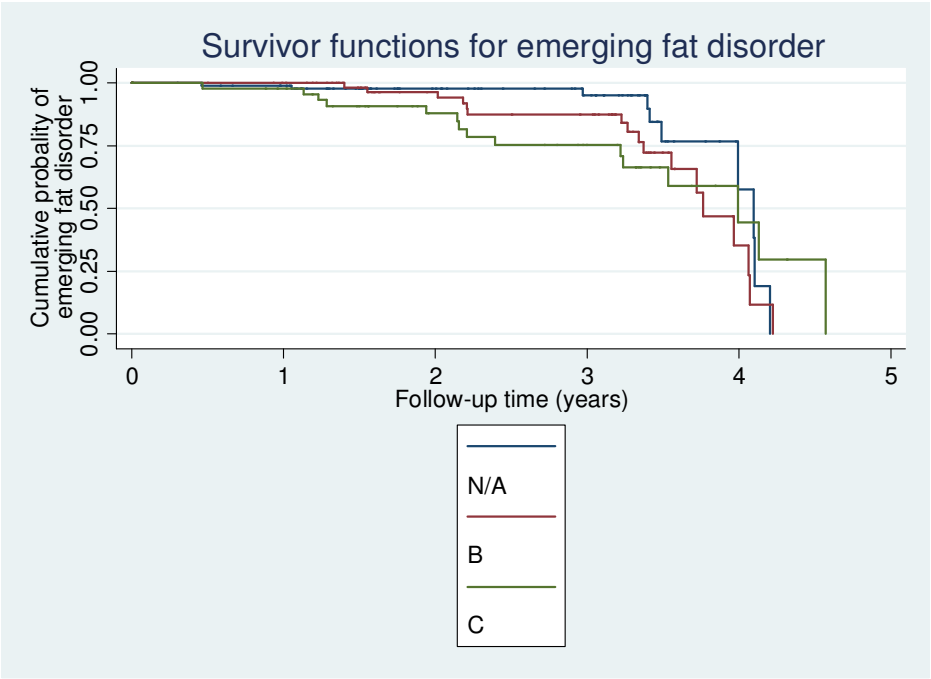
Figure F-9: Kaplan-Meir estimates for incidence lipodystrophy syndrome by (a) maximum CDC-defined clinical condition (b) nadir CDC immune status



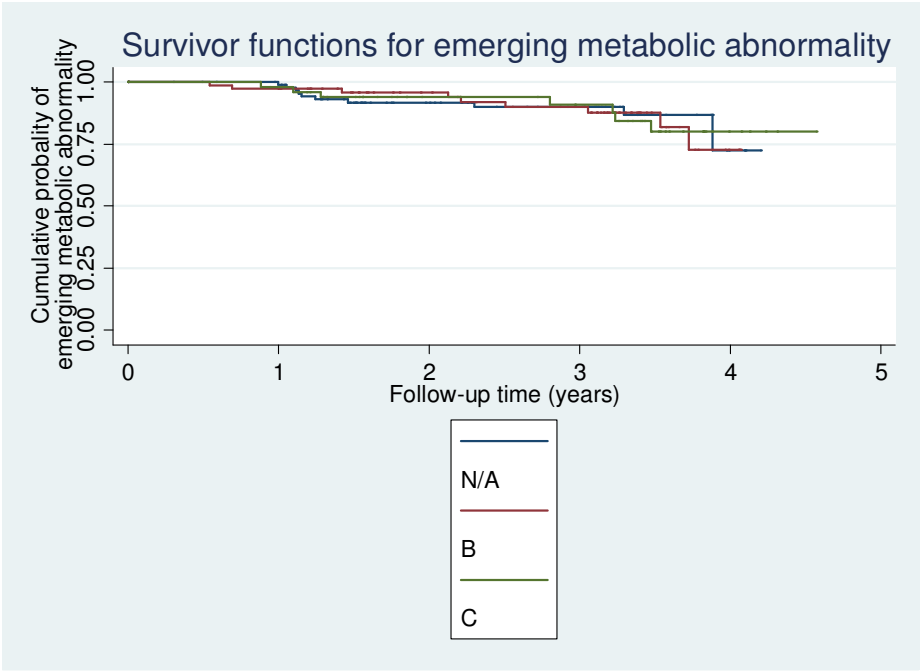
Excludes 205 subjects with continuous lipodystrophy syndrome over follow-up and17 subjects with >1 occasion of emergence/complete reversal of LS over follow-up. Log rank test: (a) $p = 0.284$, and (b) $p = 0.770$. CDC-defined clinical status - N + A: no symptoms/asymptomatic, B: moderate symptoms, C: severe symptoms.

Figure F-10: Kaplan-Meier plots for emergence of (a) body fat alterations and (b) metabolic abnormality, by maximum CDC-defined clinical condition

(a)

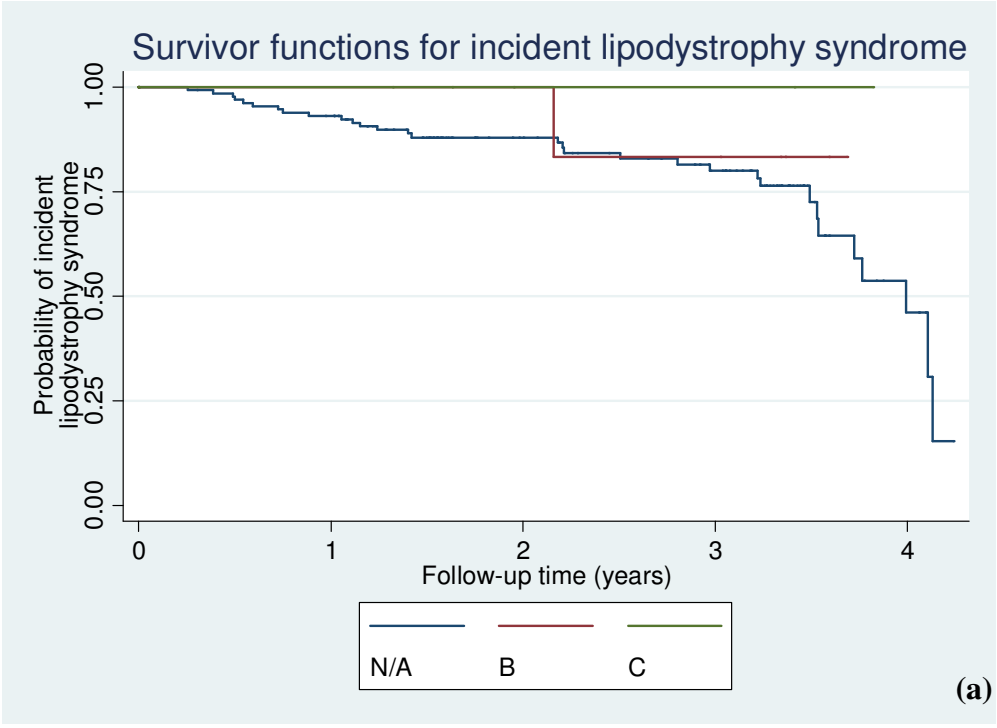


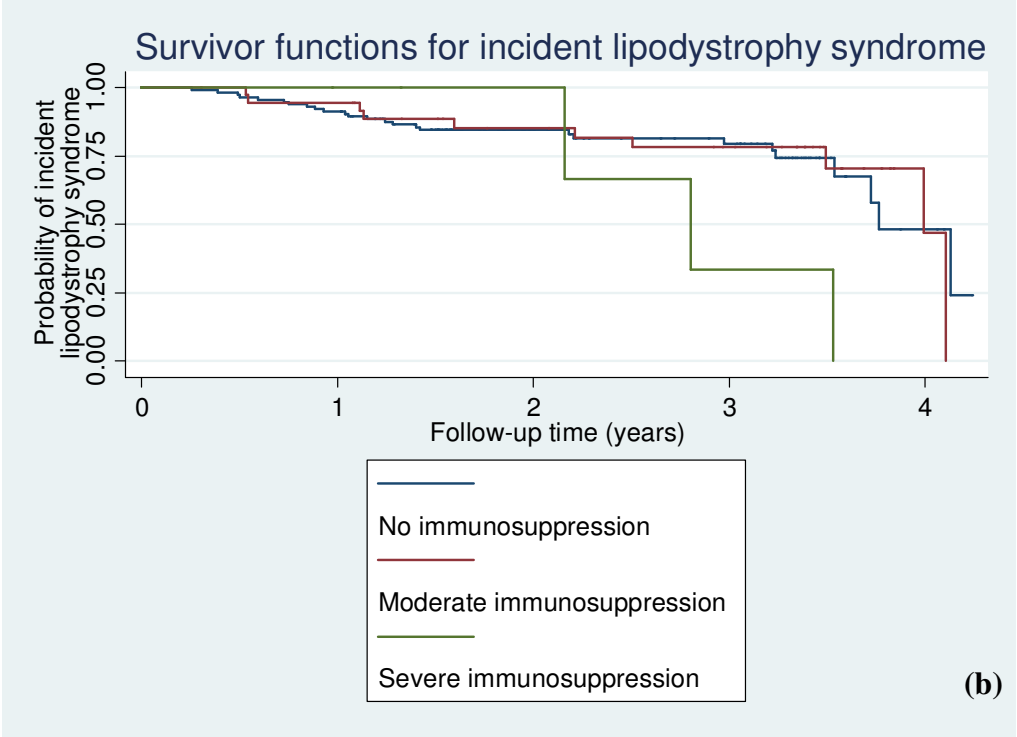
(b)



Estimates exclude 122 subjects with continuous fat alterations over follow-up and 19 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up (a), and 106 subjects with continuous metabolic abnormality over follow-up and 20 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up, (b). Log-rank test: (a) 0.0337, and (b) 0.280.

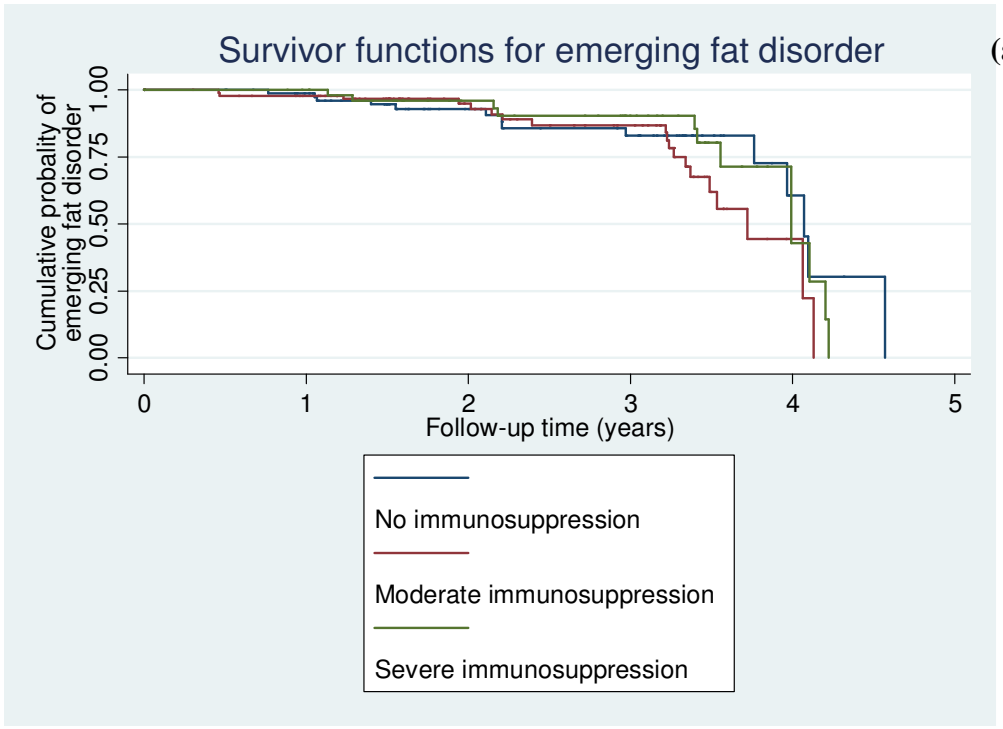
Figure F-11: Kaplan-Meir estimates for incidence lipodystrophy syndrome by recruitment CDC-defined (a) clinical status, and (b) degree of immunosuppression

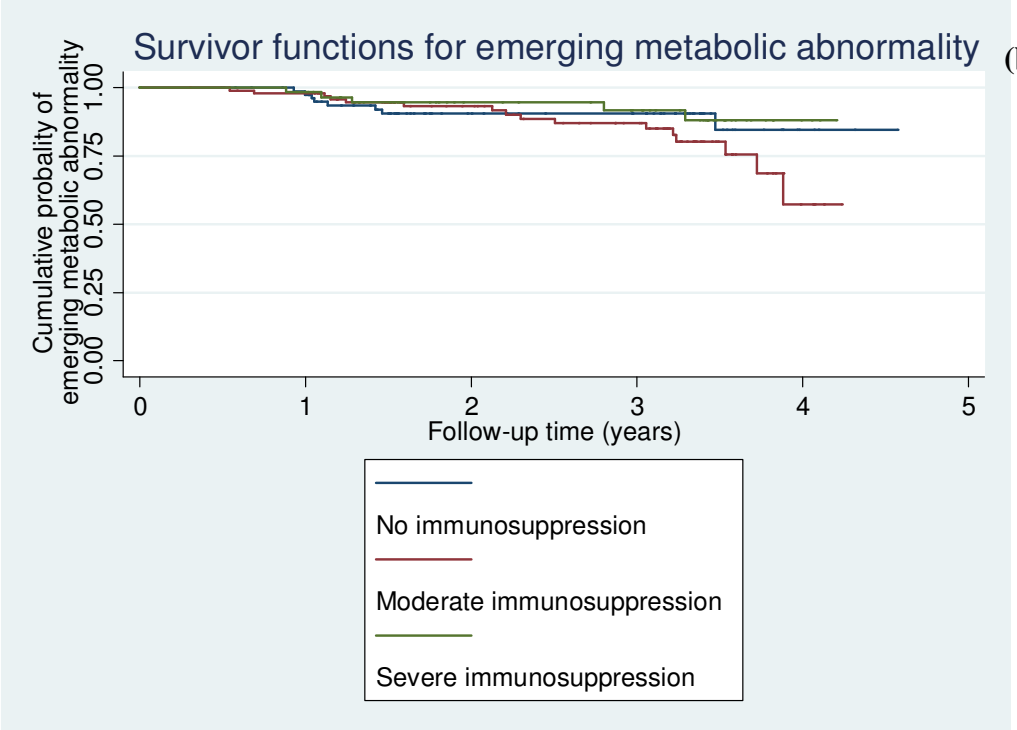




Excludes 205 subjects with continuous lipodystrophy syndrome over follow-up and17 subjects with >1 occasion of incidence/complete reversal of LS over follow-up. Log rank test: (a) $p = 0.365$, and (b) $p = 0.620$. CDC-defined clinical status - N + A: no symptoms/asymptomatic, B: moderate symptoms, C: severe symptoms.

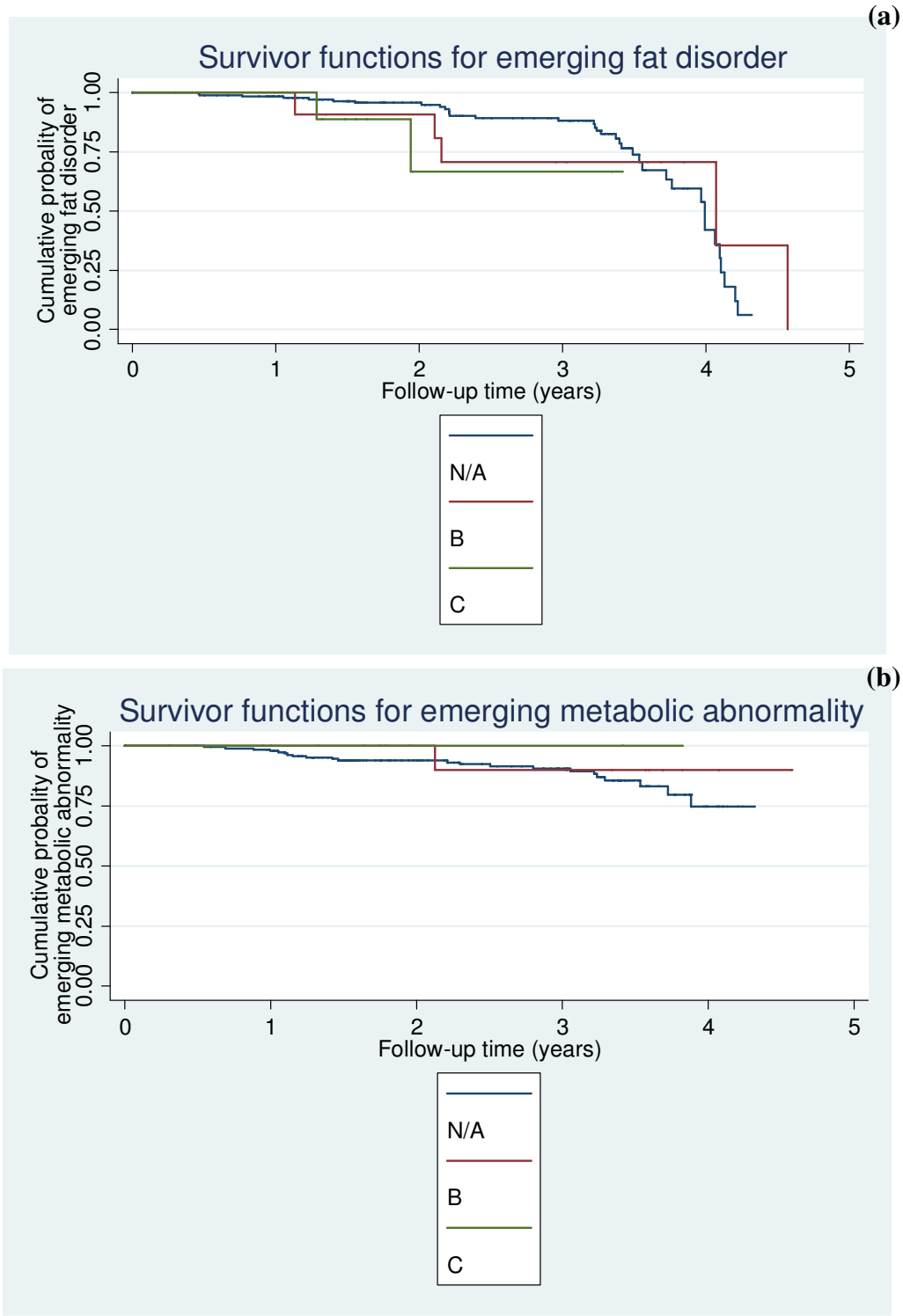
Figure F-12: Kaplan-Meier plots for emergence of (a) body fat alterations, and (b) metabolic abnormality, by nadir CDC-defined immune status





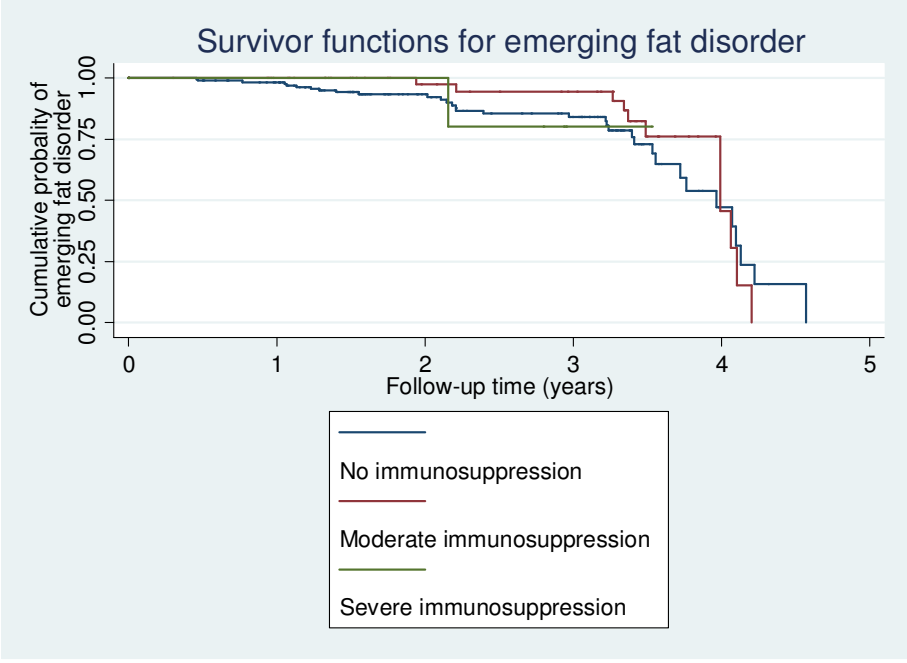
Estimates exclude 122 subjects with continuous fat alterations over follow-up and 19 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up (a), and 106 subjects with continuous metabolic abnormality over follow-up and 20 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up (b). Log-rank test: (a) $p = 0.183$, and (b) $p = 0.507$.

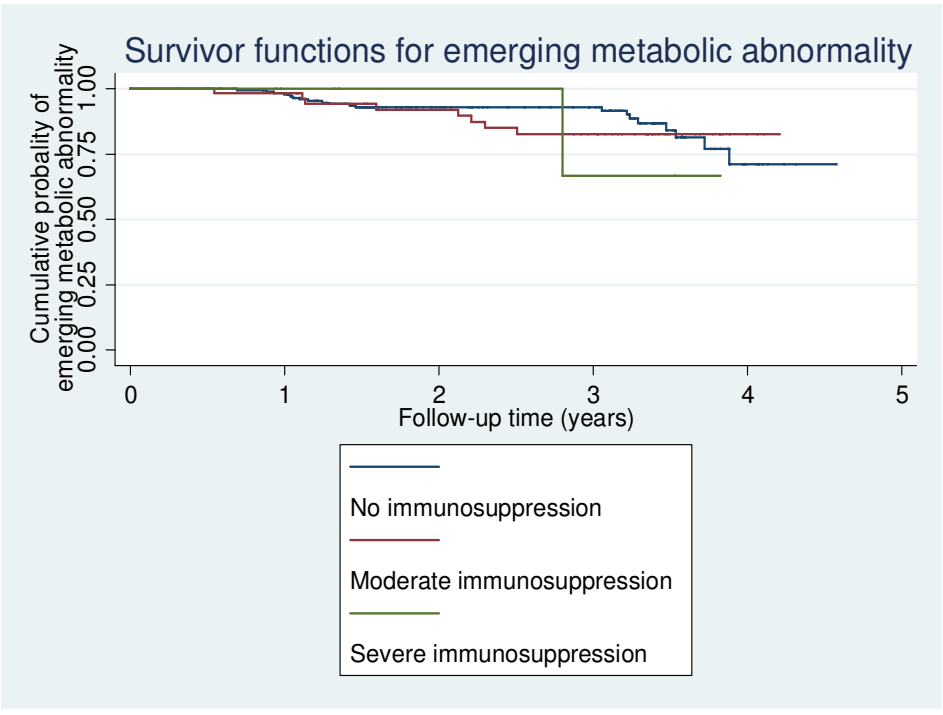
Figure F-13: Kaplan-Meier plots for emergence of (a) fat alterations, and (b) metabolic abnormality, by recruitment CDC-defined clinical condition at recruitment



Estimates exclude 122 subjects with continuous fat alterations over follow-up and 19 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up (a), and 106 subjects with continuous metabolic abnormality over follow-up and 20 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up (b). Log-rank test: (a) - p = 0.00161, and (b) – p = 0.0845.

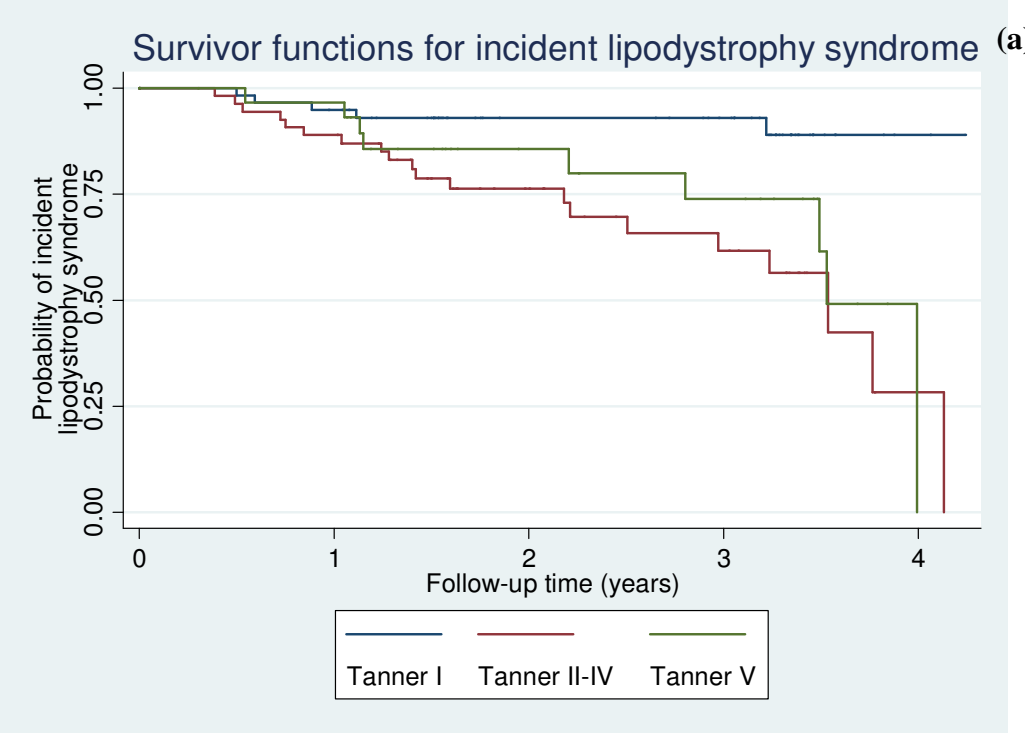
Figure F-14: Kaplan-Meier plots for emergence of (a) body fat alterations, and metabolic abnormality, by recruitment CDC-defined immune status

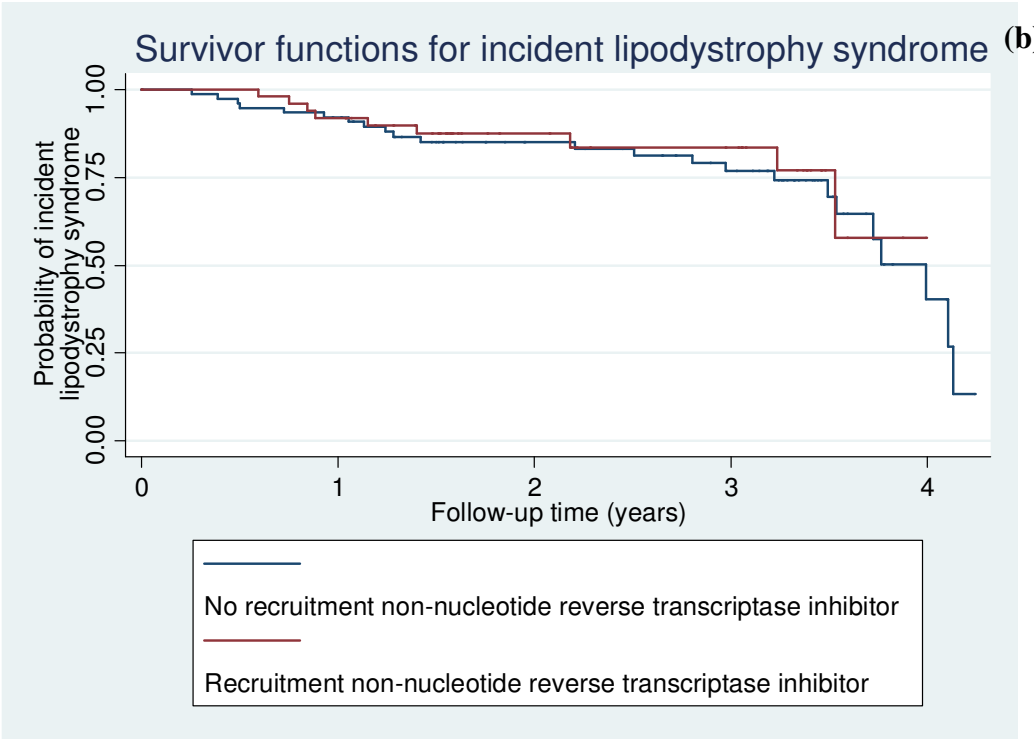




Estimates exclude 122 subjects with continuous fat alterations over follow-up and 19 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up (a), and 106 subjects with continuous metabolic abnormality over follow-up and 20 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up (b). Log-rank test: (a) - $p < 0.117$, and (b) – $p = 0.297$.

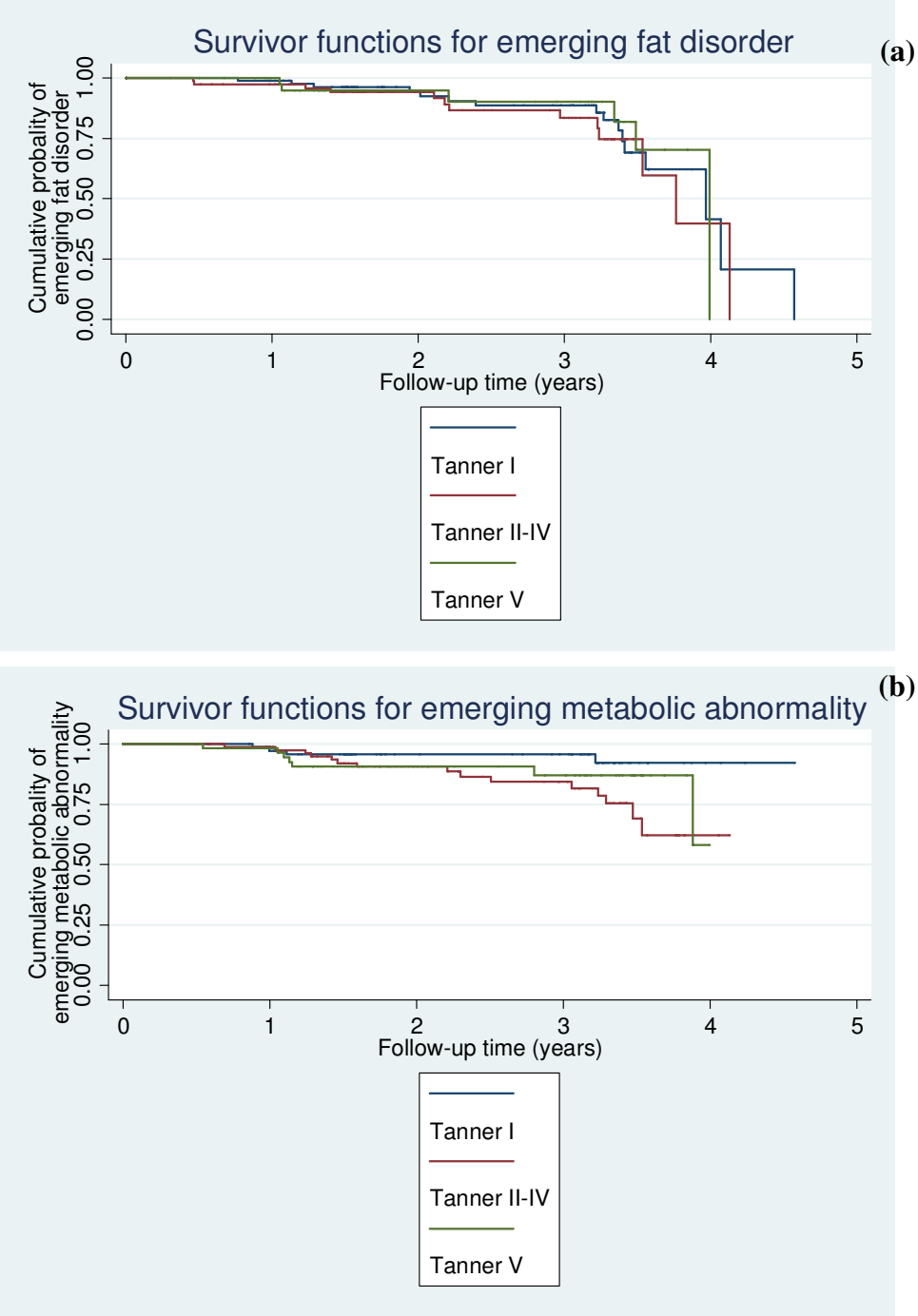
Figure F-15: Kaplan-Meir estimates for incidence lipodystrophy syndrome by (a) Tanner score for puberty at recruitment, and (b) use of non-nucleoside reverse transcriptase inhibitor use at recruitment





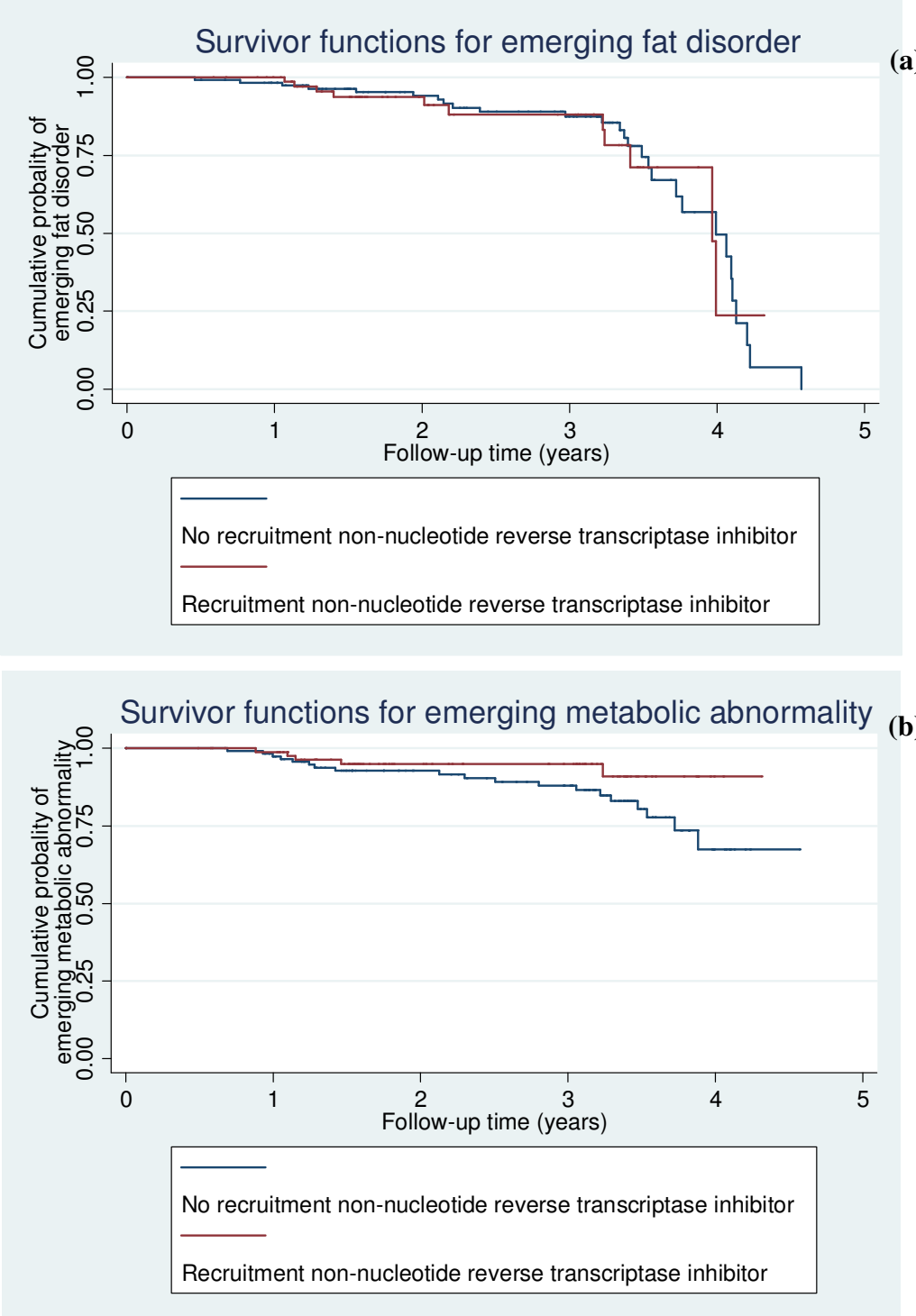
Excludes 205 subjects with continuous lipodystrophy syndrome over follow-up and17 subjects with >1 occasion of incidence/complete reversal of LS over follow-up. Log rank test: (a) $p = 0.0114$, and (b) $p = 0.974$.

Figure F-16: Kaplan-Meier plots for emergence of (a) body fat alterations, and (b) metabolic abnormality, by Tanner score for puberty at recruitment



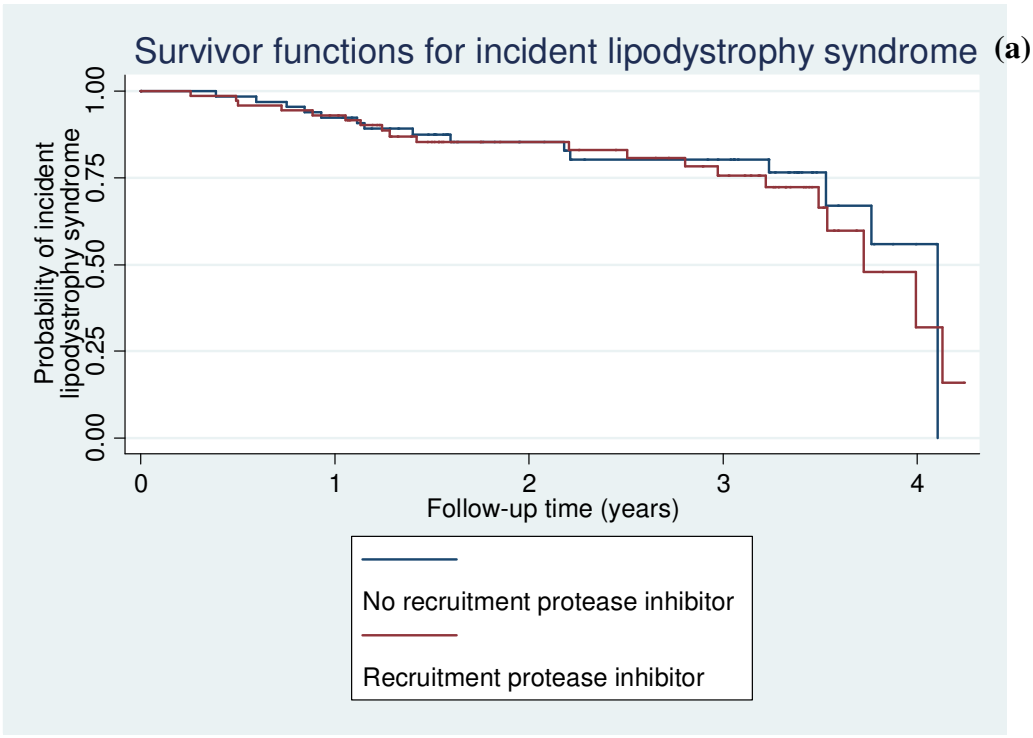
Estimates exclude 122 subjects with continuous fat alterations over follow-up and 19 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up (a), and 106 subjects with continuous metabolic abnormality over follow-up and 20 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up (b). Log-rank test: (a) - p = 0.974, and (b) - p = 0.634.

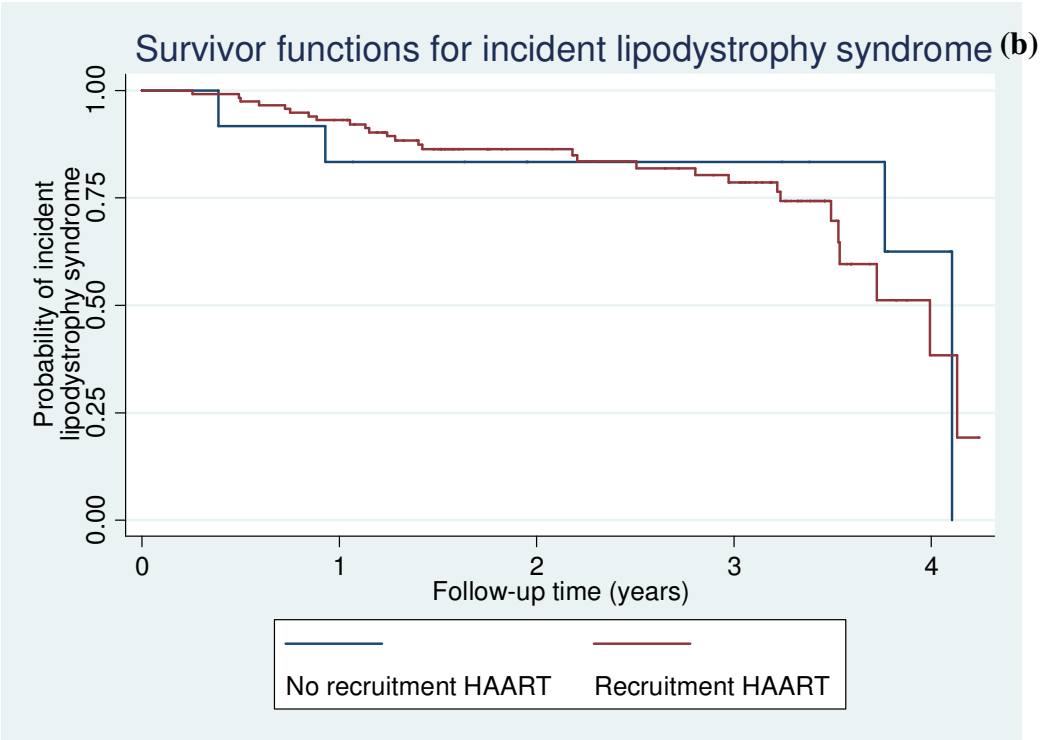
Figure F-17: Kaplan-Meier plots for emergence of (a) body fat alterations, and (b) metabolic abnormality, by use of non-nucleoside reverse transcriptase at recruitment



Estimates exclude 122 subjects with continuous fat alterations over follow-up and 19 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up (a), and 106 subjects with continuous metabolic abnormality over follow-up and 20 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up (b). Log-rank test: (a) - p = 0.0115, and (b) p = 0.155.

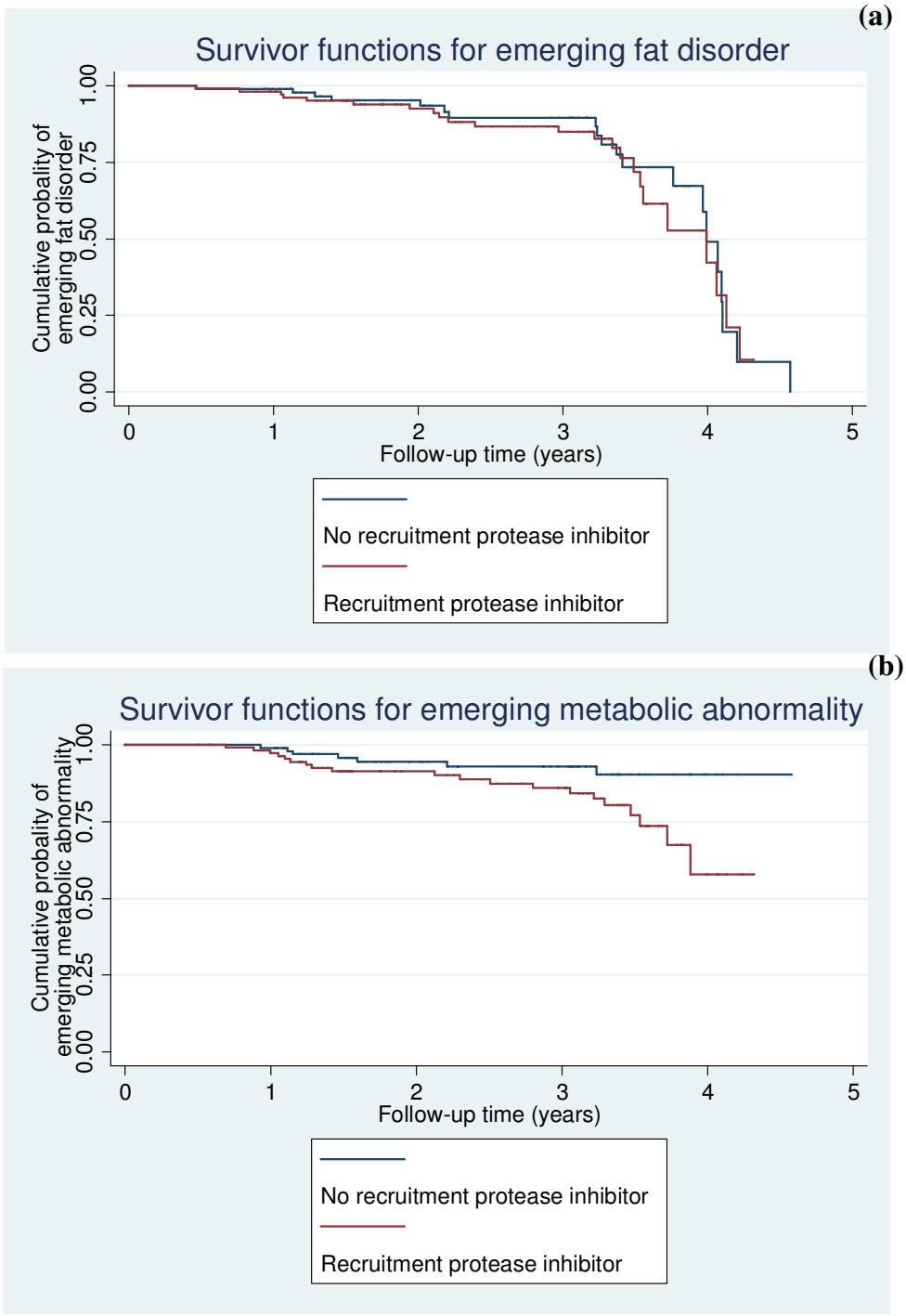
Figure F-18: Kaplan-Meir estimates for incidence lipodystrophy syndrome by use at recruitment of (a) protease inhibitors, and (b) highly active antiretroviral therapy





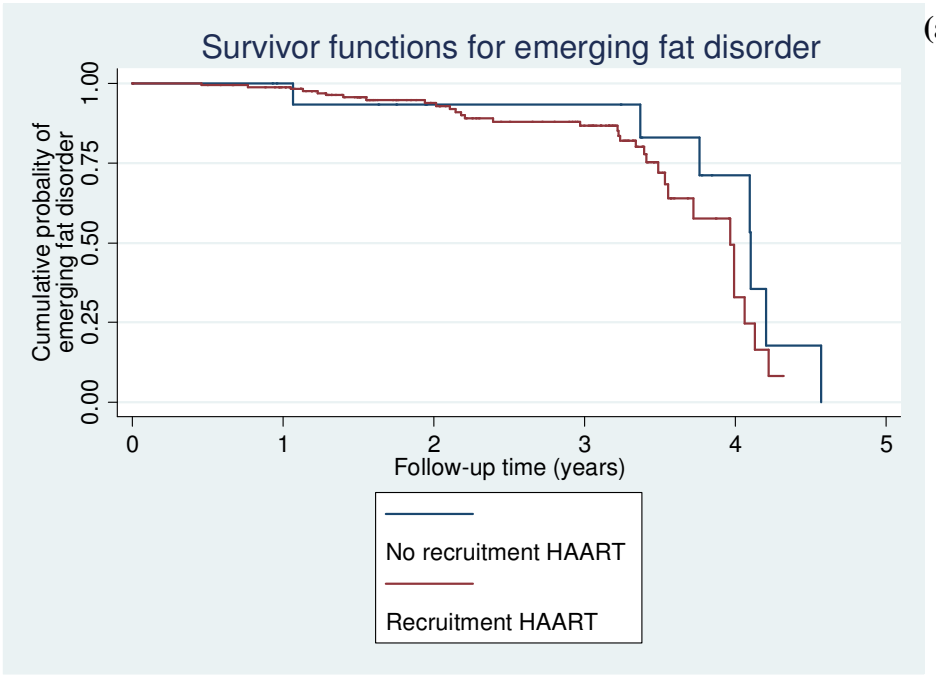
Excludes 205 subjects with continuous lipodystrophy syndrome over follow-up and17 subjects with >1 occasion of incidence/complete reversal of LS over follow-up. Log rank test: (a) $p < 0.732$, and (b) $p = 0.621$.

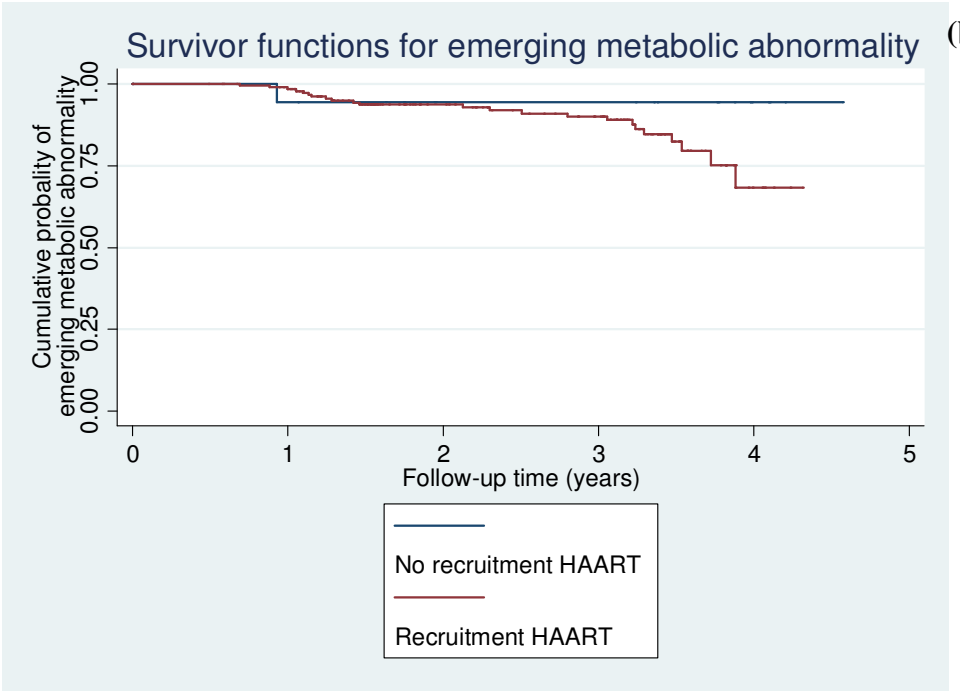
Figure F-19: Kaplan-Meier plots for emergence of fat alterations, and metabolic abnormality, by use of protease inhibitor at recruitment



Estimates exclude 122 subjects with continuous fat alterations over follow-up and 19 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up (a), and 106 subjects with continuous metabolic abnormality over follow-up and 20 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up, (b). Log-rank test: (a) $p = 0.837$, and (b) $p = 0.975$.

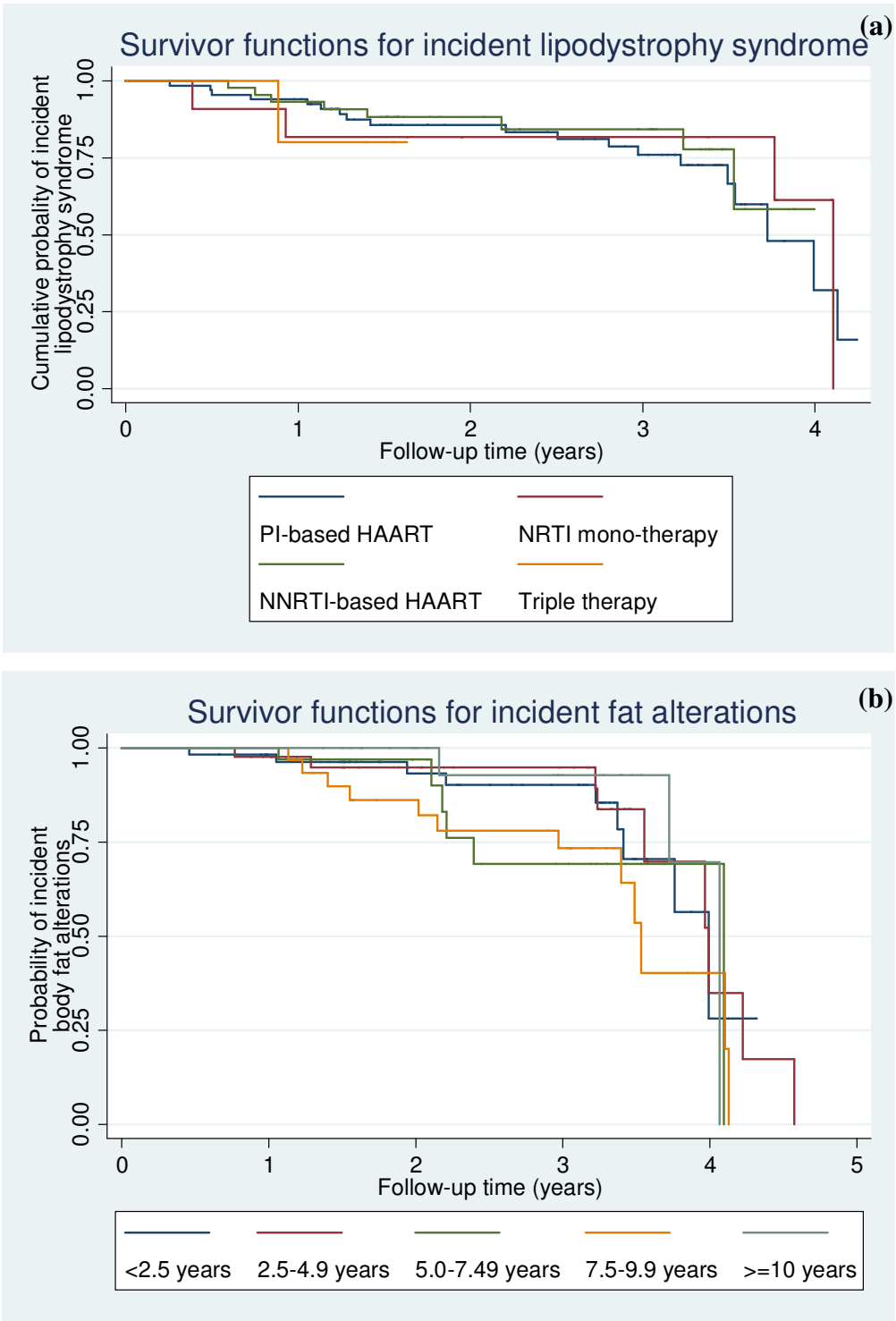
Figure F-20: Kaplan-Meier plots for emergence of fat alterations, and metabolic abnormality by use of highly active anti-retroviral therapy at recruitment





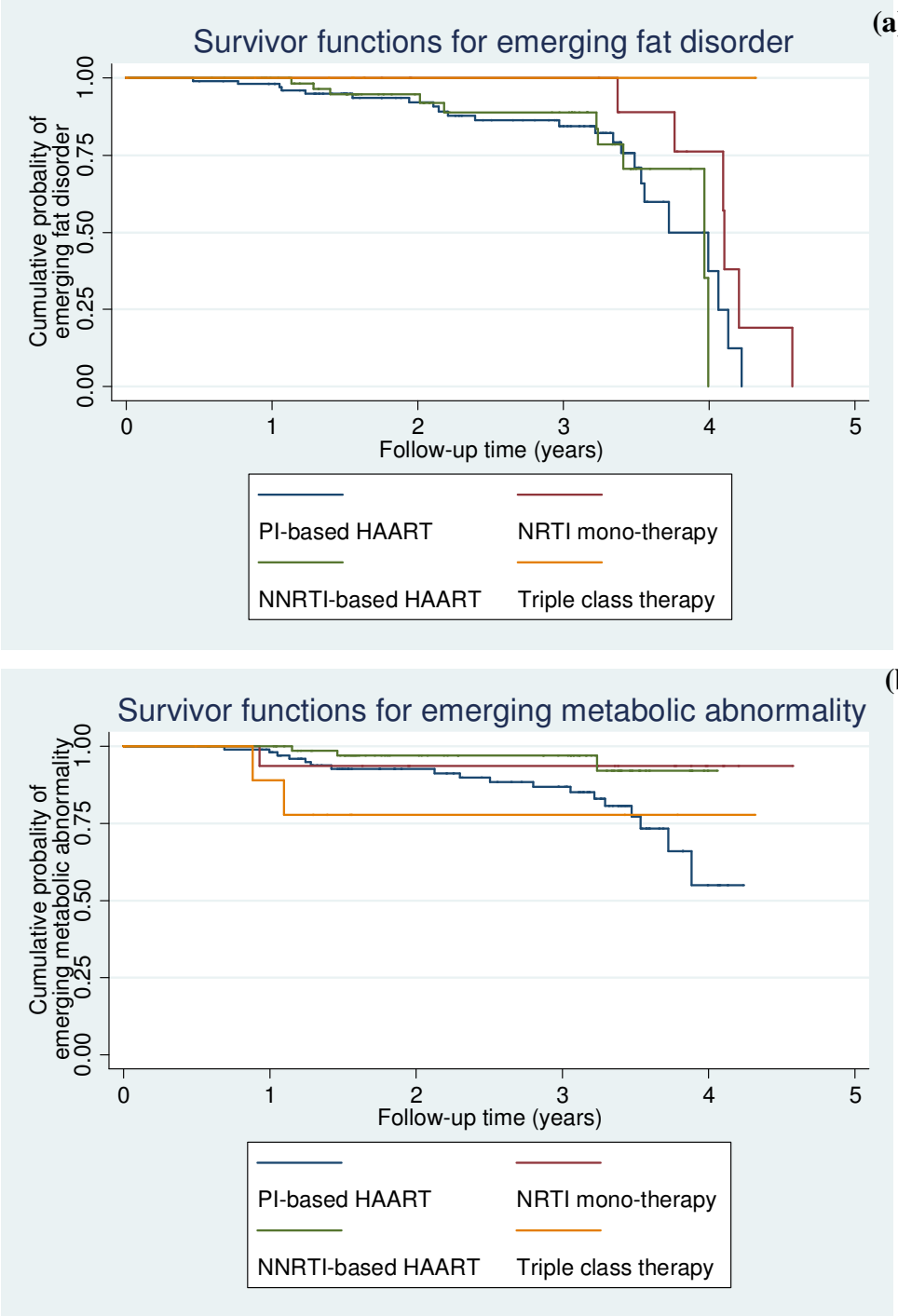
Estimates exclude 122 subjects with continuous fat alterations over follow-up and 19 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up (a), and 106 subjects with continuous metabolic abnormality over follow-up and 20 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up, (b). Log-rank test: (a) $p = 0.00309$, and (b) $p = 0.0543$.

Figure F-21: Kaplan-Meir estimates for incidence lipodystrophy syndrome by (a) antiretroviral therapy regimen at recruitment and (b) duration of ART use at recruitment



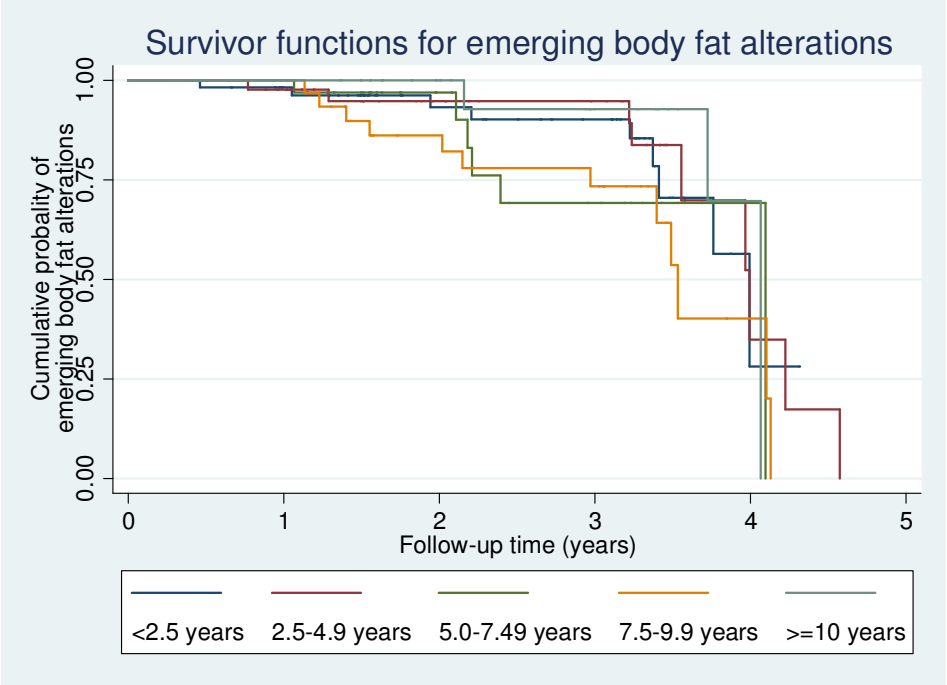
Excludes 205 subjects with continuous lipodystrophy syndrome over follow-up and17 subjects with >1 occasion of incidence/complete reversal of LS over follow-up. Log rank test: (a) $p = 0.821$, and (b) $p = 0.635$.

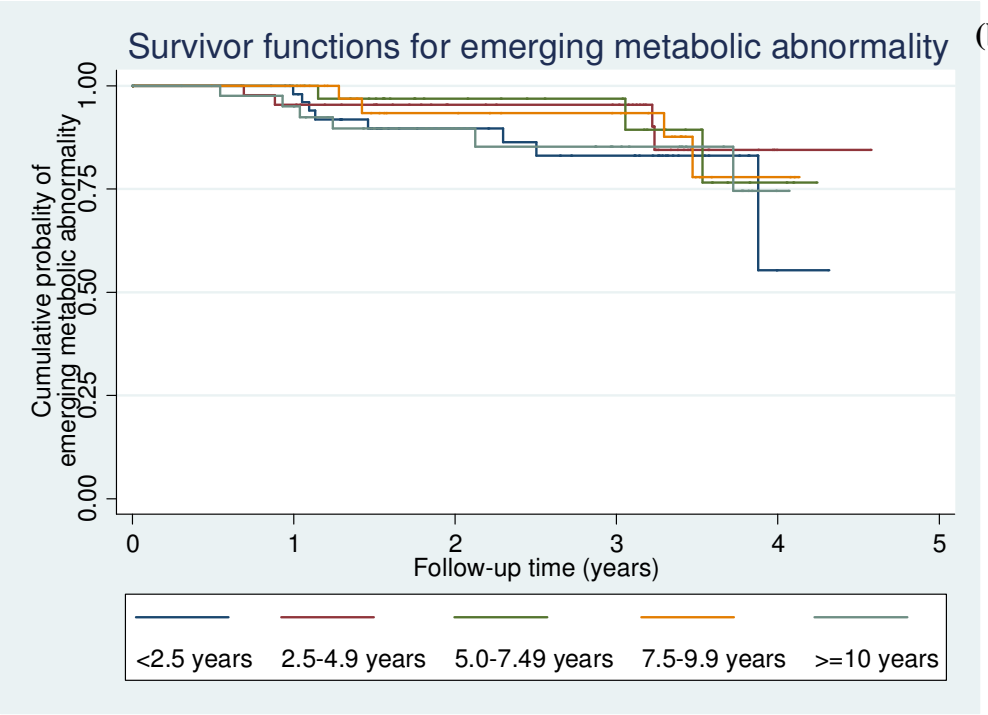
Figure F-22: Kaplan-Meier plots for emergence of fat alterations, and metabolic abnormality, by use specific antiretroviral therapy regiment at recruitment



Estimates exclude 122 subjects with continuous fat alterations over follow-up and 19 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up (a), and 106 subjects with continuous metabolic abnormality over follow-up and 20 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up. (b). Log-rank test: (a) $p = 0.00335$, and (b) $p = 0.118$.

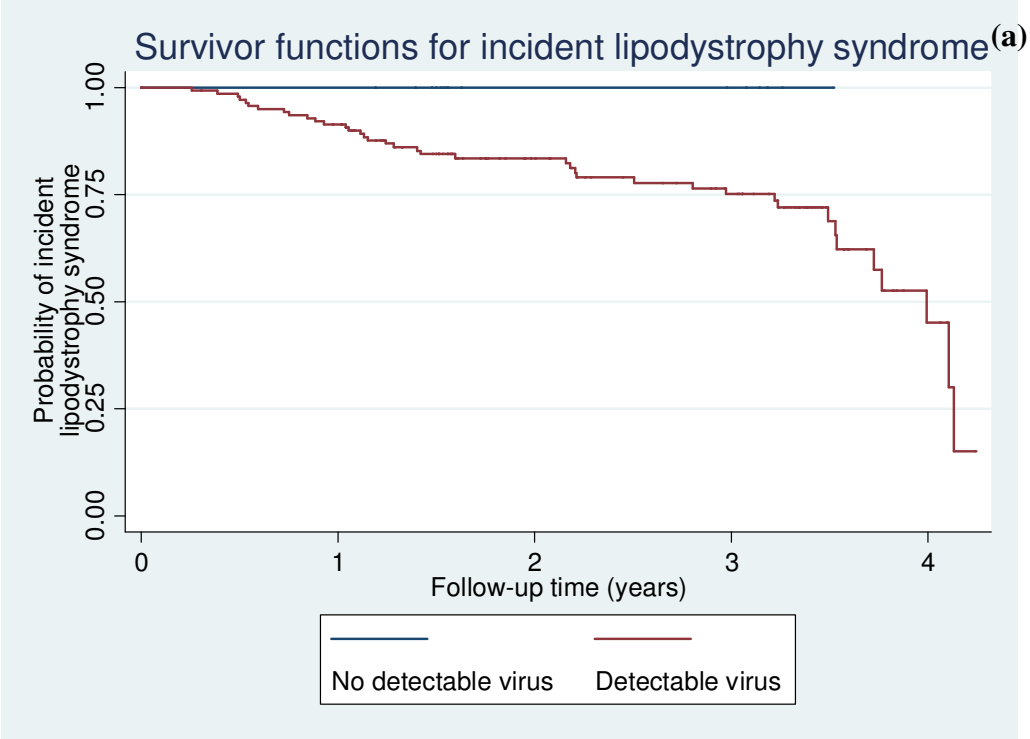
Figure F-23: Kaplan-Meier plots for emergence of fat alterations, and metabolic abnormality, by duration of ART use

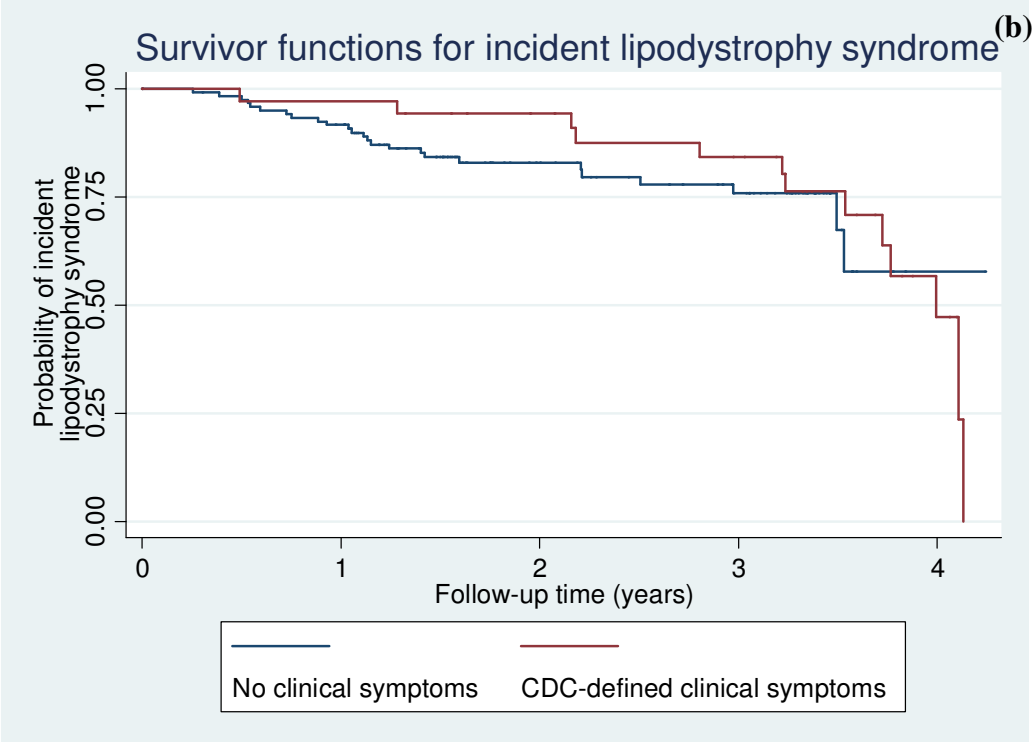




Estimates exclude 122 subjects with continuous fat alterations over follow-up and 19 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up (a), and 106 subjects with continuous metabolic abnormality over follow-up and 20 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up. (b). Log-rank test: (a) $p < 0.638$, and (b) $p = 0.698$.

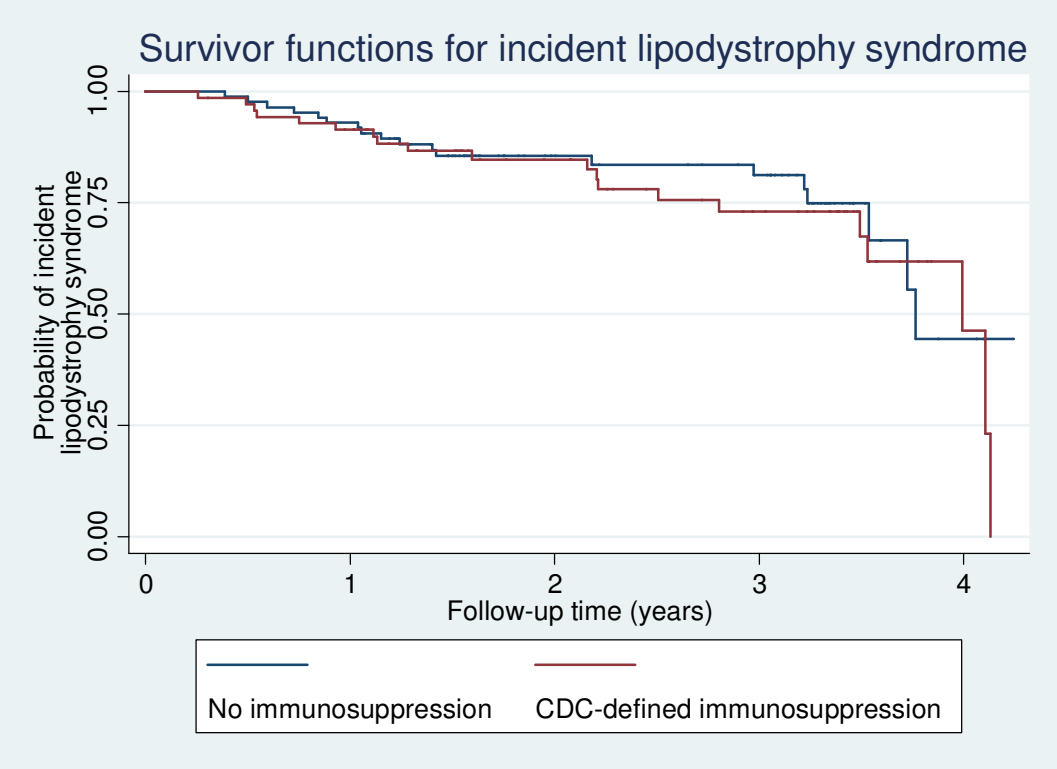
Figure F-24: Kaplan Meir plots for incidence lipodystrophy syndrome by (a) detectable viral load during follow-up, and (b) and CDC-defined clinical symptoms during follow-up





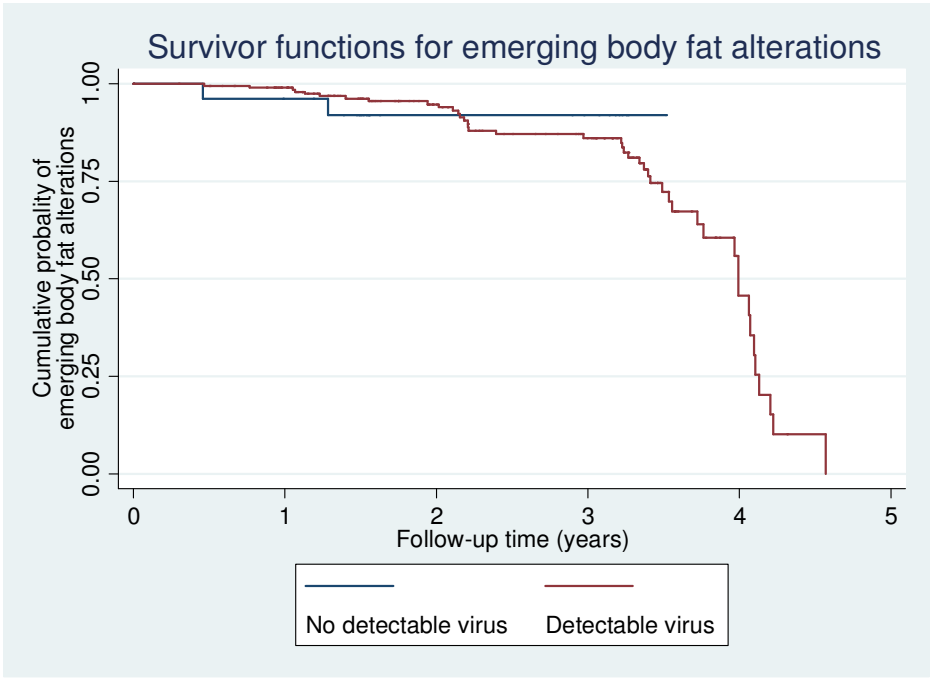
Excludes 205 subjects with continuous lipodystrophy syndrome over follow-up and17 subjects with >1 occasion of incidence/complete reversal of LS over follow-up. Log rank test: (a) $p = 0.040$, and (b) $p = 0.576$.

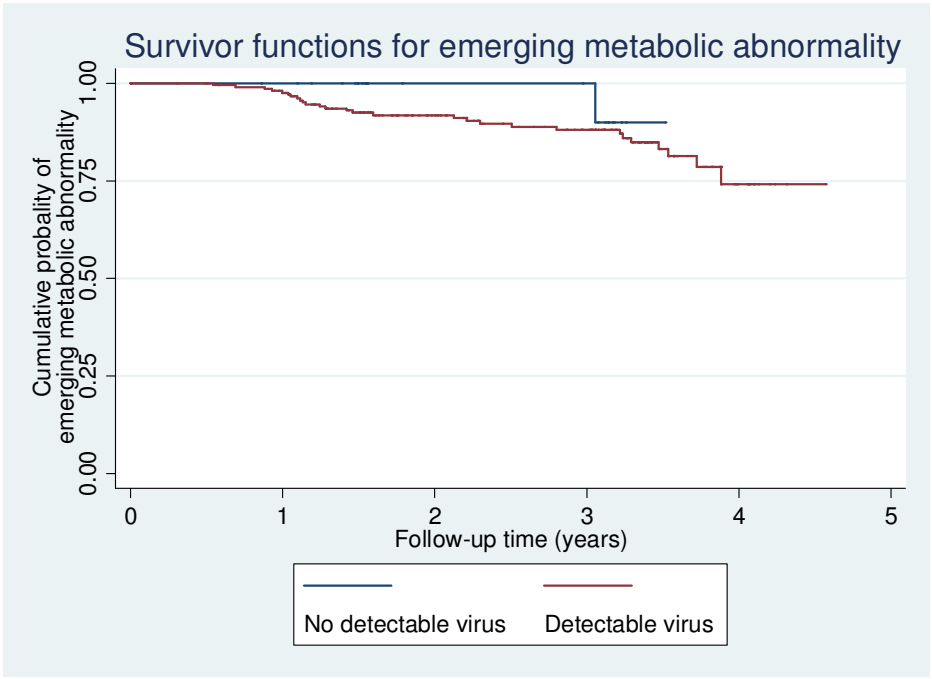
Figure F-25: Kaplan-Meir estimates for incidence lipodystrophy syndrome by CDC-defined immunosuppression during follow-up



Excludes 205 subjects with continuous lipodystrophy syndrome over follow-up and17 subjects with >1 occasion of incidence/complete reversal of LS over follow-up. Log rank test *p* = 0.046

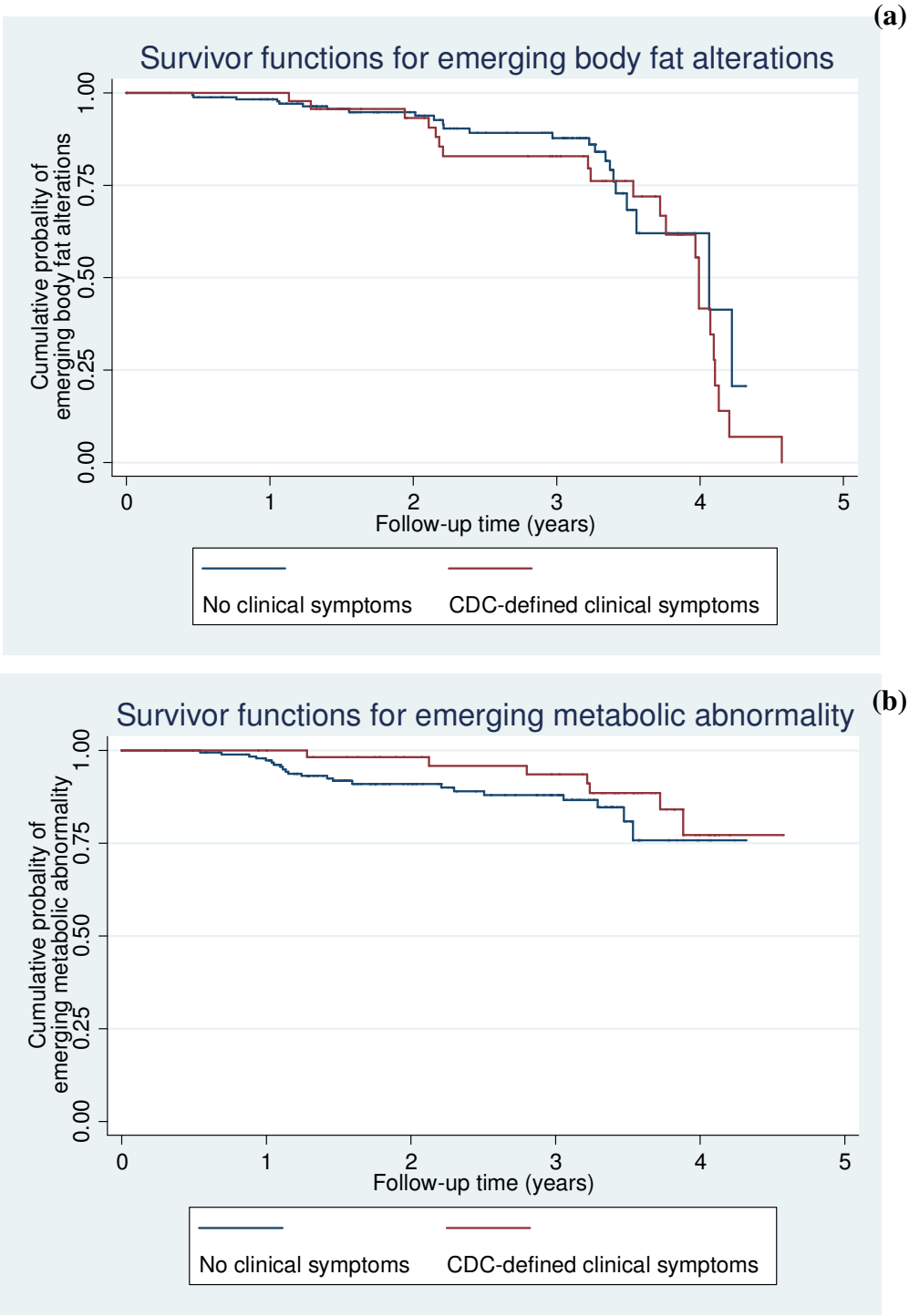
Figure F-26: Kaplan-Meier plots for emergence of fat alterations, and metabolic abnormality, by any detectable viral load during follow-up





Estimates exclude 122 subjects with continuous fat alterations over follow-up and 19 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up (a), and 106 subjects with continuous metabolic abnormality over follow-up and 20 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up. (b). Log-rank test: (a) $p < 0.001$, and (b) $p = 0.143$.

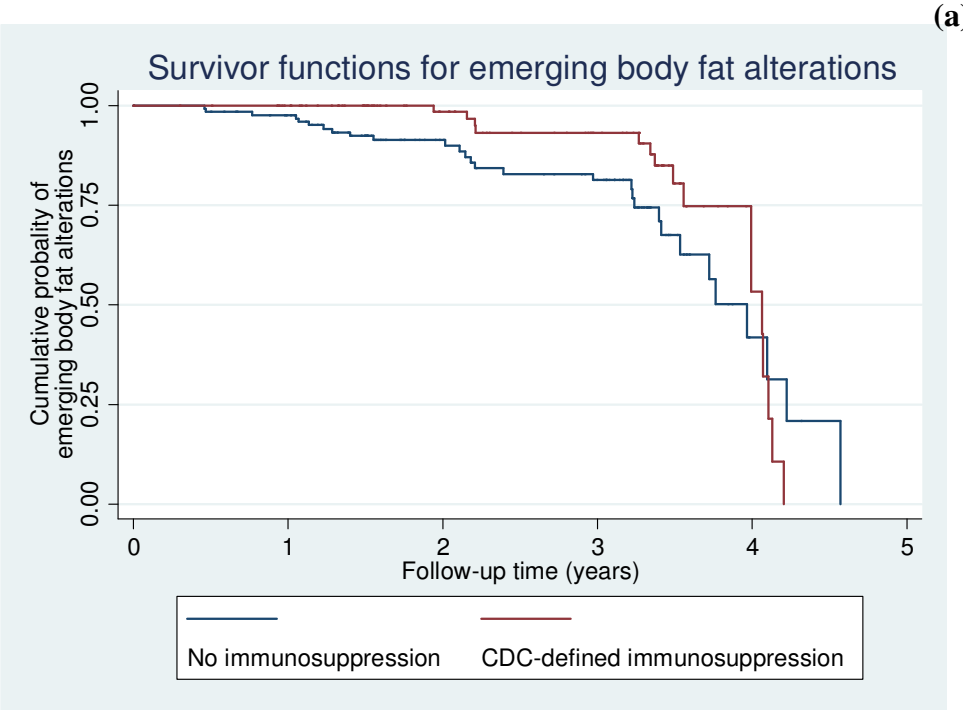
Figure F-27: Kaplan-Meier plots for emergence of fat alterations, and metabolic abnormality, by any CDC-defined clinical symptoms during follow-up



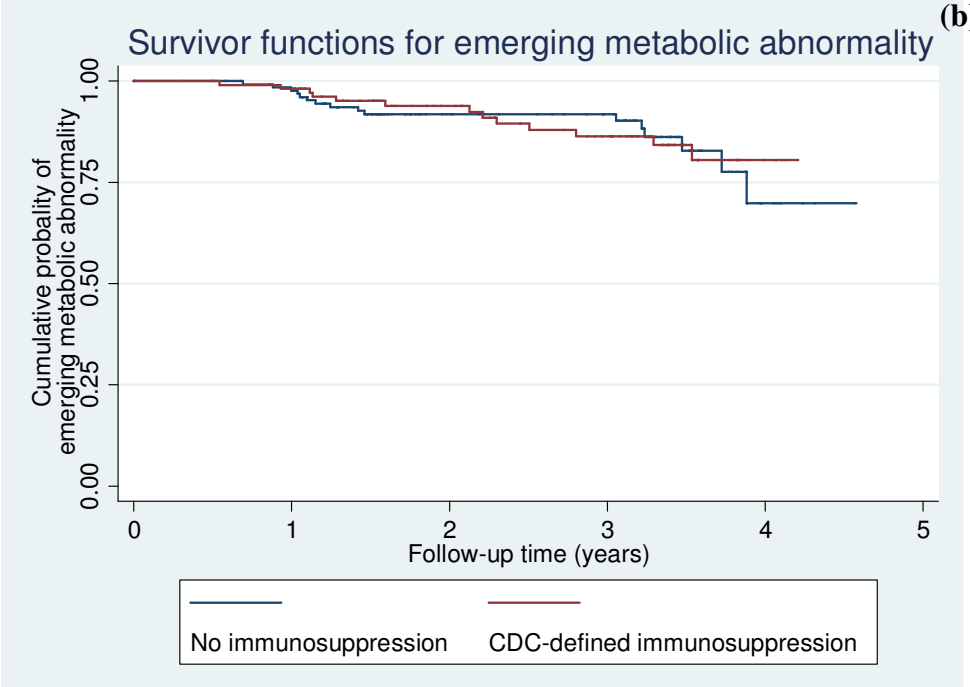
Estimates exclude 122 subjects with continuous fat alterations over follow-up and 19 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up (a), and 106 subjects with continuous metabolic abnormality over follow-up and 20 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up. (b). Log-rank test: (a) $p < 0.001$, and (b) $p = 0.040$.

Figure F-28: Kaplan-Meier plots for emergence of fat alterations, and metabolic abnormality, by any CDC-defined immunosuppression during follow-up

(a)



(b)



Estimates exclude 122 subjects with continuous fat alterations over follow-up and 19 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up (a), and 106 subjects with continuous metabolic abnormality over follow-up and 20 subjects with >1 occasion of emergence/complete regression of fat alterations over follow-up. (b). Log-rank test: (a) $p = 0.964$, and (b) $p = 0.939$.

F.6 Cox proportional hazards models with antiretroviral regimen at recruitment

Table F-4: Cox proportional hazards using ART regimen at recruitment and clinical site as a random effect

		Body fat alterations (*n* = 376)		Metabolic abnormality (*n* = 406)		Lipodystrophy syndrome (*n* = 248)	
		Adjusted hazard ratio (95% CI)	*p*-value	Adjusted hazard ratio (95% CI)	*p*-value	Adjusted hazard ratio (95% CI)	*p*-value
Age (years)*	**2-11**	1		1		1	
	12-18	1.59 (0.76, 3.31)	0.218	1.32 (0.52, 3.32)	0.558	2.01(0.89, 4.68)	0.091
Duration of ART (years)*	**<2.5**	1		1		1	
	2.5-4.9	0.71 (0.25, 1.96)	0.503	0.77 (0.22, 2.67)	0.686	0.96 (0.33, 2.83)	0.944
	5.0-7.4	1.25 (0.40, 3.89)	0.696	0.56 (0.15, 2.17)	0.403	0.52 (0.13, 2.12)	0.364
	7.5-9.9	1.28 (0.48, 3.43)	0.625	0.64 (0.19, 2.18)	0.472	1.00 (0.34, 2.96)	0.997
	≥10.0	0.35 (0.07, 1.77)	0.206	0.84 (0.23, 3.04)	0.785	-	-
ART regimen	**PI-based HAART**	1		1		1	
	NRTI-mono-therapy	0.40 (0.13, 1.24)	0.113	0.26 (0.03, 2.05)	0.199	0.87 (0.25, 2.99)	0.829
	NNRTI-based HAART	1.13 (0.49, 2.61)	0.30	0.27 (0.08, 0.95)	0.041	0.82 (0.32, 2.09)	0.677
	Triple therapy	-	-	1.21 (0.26, 5.52)	0.807	1.46 (0.17, 12.68)	0.731

Models contain random effect for clinical site of treatment. *Status at recruitment. GLOBAL test for proportional hazards: Body fat alterations *p* = 0.7011, metabolic abnormality *p* = 0.3529, lipodystrophy syndrome *p* = 0.8976

F.7 Testing proportional hazards assumptions of multivariable Cox survival models

Table F-5: Testing proportional hazards for emerging body fat alterations: recruitment factors

		χ^2 statistic	*p* – value
Age (years)*	**2-11**	Ref.	Ref.
	12-18	0.35**	0.553
Duration of ART (years)*	**<2.5**	Ref.	Ref.
	2.5-4.9	0.06**	0.808
	5.0-7.4	0.02**	0.875
	7.5-9.9	0.00**	0.989
	≥10.0	1.32**	0.250
GLOBAL		1.74***	0.884

***Status at recruitment. **1 degree of freedom. ***5degrees of freedom**

Table F-6: Testing proportional hazards for emerging body fat alterations: follow-up factors

		χ^2 statistic	*p* – value
Age (years)*	**2-11**	Ref.	Ref.
	12-18	0.57**	0.452
Duration of ART (years)*	**<2.5**	Ref.	Ref.
	2.5-4.9	0.08**	0.780
	5.0-7.4	0.00**	0.964
	7.5-9.9	0.04**	0.850
	≥10.0	1.35**	0.245
Immunosuppression during follow-up	**No immunosuppression**	Ref.	Ref.
	Immunosuppression	2.29**	0.130
GLOBAL		4.03***	0.673

***Status at recruitment. **1 degree of freedom. ***6 degrees of freedom**

Table F-7: Testing proportional hazards for emerging metabolic abnormality: recruitment factors

		χ^2 statistic	*p* – value
Age (years)*	**2-11**	Ref.	Ref.
	12-18	0.63**	0.428
Duration of ART (years)*	**<2.5**	0.00**	0.970
	2.5-4.9	Ref.	Ref.
	5.0-7.4	0.09**	0.769
	7.5-9.9	0.09**	0.769
	≥10.0	0.01**	0.936
Body Mass index*	**Severely underweight**	Ref.	Ref.
	Underweight	1.36**	0.244
	Health weight	2.53**	0.111
	Overweight	0.13**	0.721
	Obese	-	-
PI*	**No use**	Ref.	Ref.
	Use	0.61**	0.434
GLOBAL		6.70***	0.668

***Status at recruitment.** 1 degree of freedom. ***9 degrees of freedom**

Table F-8: Testing proportional hazards for emerging metabolic abnormality: follow-up factors

		χ^2 statistic	*p* – value
Age (years)*	**2-11**	Ref.	Ref.
	12-18	0.55**	0.451
Duration of ART (years)*	**<2.5**	Ref.	Ref.
	2.5-4.9	0.03**	0.852
	5.0-7.4	0.35**	0.555
	7.5-9.9	0.10**	0.747
	≥10.0	0.02**	0.899
Body Mass index*	**Severely underweight**	Ref.	Ref.
	Underweight	1.02**	0.313
	Health weight	2.17**	0.141
	Overweight	0.01**	0.913
	Obese	0.00**	1.00
PI*	**No use**	Ref.	Ref.
	Use	0.04**	0.846
Immuno-suppression during follow-up	**No immunosuppression**	Ref.	Ref.
	Immunosuppression	1.31**	0.253
GLOBAL		6.27***	0.855

***Status at recruitment. **1 degree of freedom. ***11 degrees of freedom**

Table F-9: Testing proportional hazards for emerging lipodystrophy syndrome: recruitment factors

		χ^2 statistic	*p* – value
Age(years)*	**2-11**	Ref.	Ref.
	12-18	1.45**	0.229
Duration of ART (years)*	**<2.5**	Ref.	Ref.
	2.5-4.9	0.05**	0.829
	5.0-7.4	0.22**	0.638
	7.5-9.9	0.78**	0.377
	≥10.0	0.13**	0.723
Ethnicity	**Black**	Ref.	Ref.
	White	0.15**	0.697
	Other	0.01**	0.927
Maximum CDC-defined clinical status	**N/A**	Ref.	Ref.
	B	0.80**	0.372
	C	0.13**	0.717
GLOBAL		4.90***	0.843

***Status at recruitment. **1 degree of freedom.** *9 degrees of freedom**

Table F-10: Testing proportional hazards for emerging lipodystrophy syndrome: recruitment ART regimen

		Body fat alterations		Metabolic abnormality		Lipodystrophy syndrome	
		χ^2 statistic	*p*-value	χ^2 statistic	*p*-value	χ^2 statistic	*p*-value
Age (years)*	**2-11**						
	12-18	0.00**	0.995	1.09**	0.297	1.14**	0.286
Duration of	**<2.5**						
ART (years)*	**2.5-4.9**	0.05**	0.825	0.33**	0.567	0.73**	0.391
	5.0-7.4	0.02**	0.882	0.97**	0.325	0.03**	0.859
	7.5-9.9	0.19**	0.661	0.51**	0.474	0.84**	0.360
	≥10.0	1.26**	0.261	0.26**	0.611	0.02**	0.894
ART regimen	**PI-based HAART**	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
	NRTI-mono-therapy	0.59**	0.441	3.27**	0.070	0.32**	0.572
	NNRTI-based HAART	2.00**	0.158	0.09**	0.760	0.01**	0.905
	Triple therapy	-	-	3.09**	0.079	0.00**	
GLOBAL		4.66***	0.701	8.88#	0.8352	3.52#	0.8976

***Status at recruitment. **1 degree of freedom. *** 7degrees of freedom. #8 degrees of freedom**

Appendix G Appendix to Chapter 7

G.1 Median lipid concentrations in subjects with incident body fat alterations

Table G-1: Comparison of median serum concentrations of lipids in subjects with incident body fat alterations at recruitment and end of follow-up by sex and age (*n* = 62)

		Male			Female		
		Recruitment	End of follow-up	*p* -value	Recruitment	End of follow-up	*p* -value
Fasting triglyceride (mg/dL)	**2-11 years**	65 (41, 122)	125 (50, 289)	1.000	64 (55, 97)	93 (51, 164)	1.000
	12-18 years	179 (83, 253)	107 (58, 133)	0.006	87 (54, 111)	122 (79, 173)	0.180
Total cholesterol (mg/dL)	**2-11 years**	186 (165, 217)	189 (167, 206)	1.000	178 (166, 198)	190 (155, 203)	0.648
	12-18 years	172 (131, 197)	162 (118, 197)	0.791	170 (121, 197)	166 (122, 166)	0.629
LDL cholesterol (mg/dL)	**2-11 years**	83 (78, 112)	67 (57, 110)	1.00	99 (82, 120)	99 (85, 126)	1.000
	12-18 years	90 (73, 109)	92 (67, 122)	0.581	80 (69, 100)	89 (70, 111)	0.454
Non-HDL cholesterol (mg/dL)	**2-11 years**	112 (89, 131)	136 (134, 151)	0.453	115 (98, 159)	114 (89, 150)	0.648
	12-18 years	131 (105, 152)	109 (87, 148)	0.607	98 (85, 140)	126 (98, 147)	0.607
HDL cholesterol (mg/dL)	**2-11 years**	59 (46, 63)	48 (37, 73)	0.453	64 (51, 67)	61 (52, 72)	1.000
	12-18 years	39 (35, 52)	38 (30, 61)	0.607	50 (38, 71)	40 (39, 58)	0.454

Interquartile range in brackets. Males 2-11 years old: *n* = 10. Males 12-18 years old: *n* =16. Females 2-11 years old: *n* = 19. Females 12-18 years old: *n* = 17

Table G-2: Univariable 3-level multilevel models of serum concentrations

		Total cholesterol		LDL-cholesterol		Non-HDL cholesterol		HDL cholesterol		Fasting triglyceride	
		Beta (95% CI)	*p*-value	Beta (95% CI)	*p*-value	Beta (95% CI)	*p*-value	Beta (95% CI)	*p*-value	Beta (95% CI)	*p*-value
Recruitment lipodystrophy	**Absent**	Comparison		Comparison		Comparison		Comparison		Comparison	
	Present	26.33 (17.78, 34.88)	<0.001	18.47 (11.90, 25.03)	<0.001	26.14 (18.09, 34.19)	<0.001	-0.05 (-3.78, 3.68)	0.979	41.40 (26.00, 56.80)	<0.001
Any LS over follow-up	**Never**	Comparison		Comparison		Comparison		Comparison		Comparison	
	Any	28.77 (18.80, 38.75)	<0.001	20.50 (12.76, 28.25)	<0.001	28.17 (18.88, 37.46)	<0.001	-0.20 (-4.49, 4.10)	0.928	45.89 (27.52, 64.27)	<0.001
Age at recruitment	**<12 years**	Comparison		Comparison		Comparison		Comparison		Comparison	
	12-18 years	-17.20 (-23.98, -10.41)	<0.001	-10.90 (-16.24, -5.56)	<0.001	-10.15 (-16.72, -3.59)	0.002	-5.71 (-8.59, -2.83)	<0.001	6.43 (-6.50, 19.35)	0.330
Ethnicity	**Black**	Comparison		Comparison		Comparison		Comparison		Comparison	
	White	1.72 (-7.44, 10.88)	0.713	1.94 (-5.07, 8.94)	0.588	8.97 (0.31, 17.63)	0.042	-7.45 (-11.49, -3.42)	<0.001	44.68 (29.28, 60.07)	<0.001
	Other	-5.17 (-23.37, 13.03	0.578	-4.06 (-18.24, 10.11)	0.574	-0.51 (-17.32, 16.29)	0.952	-5.11 (-12.86, 2.64)	0.196	22.92 (-9.28, 55.13)	0.163
Sex	**Male**	Comparison		Comparison		Comparison		Comparison		Comparison	
	Female	3.20 (-3.84, 10.24)	0.373	2.07 (-3.43, 7.60)	0.461	-1.14 (-7.73, 5.44)	0.733	3.91 (1.01, 6.81)	0.008	-17.12 (-29.86, -4.36)	0.009
Detectable viral load at recruitment*	**<50 copies/ml**	Comparison		Comparison		Comparison		Comparison		Comparison	
	≥50 copies/ml	-21.45 (-31.74, -11.16)	<0.001	-14.82 (-22.75, -6.88)	<0.001	-19.09 (-29.52, -8.65)	<0.001	-5.24 (-10.14, -0.33)	0.037	-18.02 (-39.24, 3.20)	0.096
Any detectable viral load over follow-up**	**Never**	Comparison		Comparison		Comparison		Comparison		Comparison	
	Any	-25.52 (-36.46, -14.57)	<0.001	-16.83 (-25.30, -8.35)	<0.001	-21.25 (-32.24, -10.26)	<0.001	-6.59 (-11.71, -1.47)	0.012	-13.36 (-35.88, 9.17)	0.245
Recruitment immuno-suppression	**None**	Comparison		Comparison		Comparison		Comparison		Comparison	
	Moderate	-12.08 (-20.86, -3.30)	0.007	-7.01 (-13.84, -0.17)	0.044	-7.20 (-15.40, 1.10)	0.086	-4.19 (-7.83, -0.56)	0.024	-5.49 (-21.51, 10.53)	0.502
	Severe	-9.55 (-28.30, 9.20)	0.318	0.65 (-14.40, 15.71)	0.932	5.81 (-12.25, 23.88)	0.528	-6.80 (-14.80, 1.20)	0.096	16.53 (-18.36, 51.42)	0.353
Immuno-suppression during follow-up	**None**	Comparison		Comparison		Comparison		Comparison		Comparison	
	Any	-14.48 (-21.49, -7.46)	<0.001	-8.67 (-14.17, -3.16)	0.002	-11.22 (-17.82, -4.62)	0.001	-3.06 (-6.03, -0.10)	0.043	-11.93 (-24.97, 1.11)	0.073
Nadir immuno-suppression	**None**	Comparison		Comparison		Comparison		Comparison		Comparison	
	Moderate	-4.43 (-12.44, 3.57)	0.278	-2.12 (-8.35, 4.11)	0.505	-1.67 (-9.12, 5.78)	0.661	-1.46 (-4.77, 1.86)	0.389	5.90 (-8.67, 20.47)	0.427
	Severe	0.74 (-8.41, 9.88)	0.875	-0.88 (-8.04, 6.28)	0.809	3.24 (-5.33, 11.82)	0.459	-2.46 (-6.27, 1.35)	0.206	14.19 (-2.67, 31.05)	0.099
Clinical condition at recruitment	**N+A**	Comparison		Comparison		Comparison		Comparison		Comparison	
	B	3.24 (-13.81, 20.30)	0.709	3.22 (-9.82, 16.28)	0.628	5.18 (-10.52, 20.88)	0.518	-1.72 (-8.58, 5.15)	0.624	0.65 (-30.04, 31.33)	0.967
	C	-4.28 (-25.13, 16.56)	0.687	3.41 (-14.06, 20.89)	0.702	5.83 (-15.02, 26.67)	0.584	0.86 (-8.75, 10.52)	0.857	27.29 (-13.81, 68.38)	0.193

		Total cholesterol		LDL-cholesterol		Non-HDL cholesterol		HDL cholesterol		Fasting triglyceride	
		Beta (95% CI)	*p*-value	Beta (95% CI)	*p*-value	Beta (95% CI)	*p*-value	Beta (95% CI)	*p*-value	Beta (95% CI)	*p*-value
Any clinical symptoms over follow-up	**None**	Comparison		Comparison		Comparison		Comparison		Comparison	
	Any	-4.69 (-14.08, 4.71)	0.328	0.80 (-6.48, 8.07)	0.830	-3.16 (-12.06, 5.74)	0.486	-0.14 (-4.13, 3.85)	0.945	-9.69 (-27.01, 7.64)	0.273
Maximum clinical status	**N+A**	Comparison		Comparison		Comparison		Comparison		Comparison	
	B	6.68 (-1.55, 14.92)	0.111	3.72 (-2.66, 10.10)	0.253	6.24 (-1.38, 13.87)	0.108	0.24 (-3.10, 3.58)	0.888	16.54 (1.32, 31.75)	0.033
	C	6.97 (-2.52, 16.47)	0.150	7.42 (0.06, 14.77)	0.048	9.22 (0.43, 18.02)	0.040	-1.53 (-5.39, 2.33)	0.437	20.12 (2.31, 37.92)	0.027
Body mass index at recruitment	**Healthy**	Comparison		Comparison		Comparison		Comparison		Comparison	
	Severely underweight	13.25 (3.83, 22.66)	0.006	10.63 (3.34, 17.92)	0.004	6.39 (-2.45, 15.24)	0.157	5.17 (1.28, 9.06)	<0.001	-9.90 (-27.29, 7.48)	0.264
	Underweight	0.98 (-7.34, 9.29)	0.818	-0.70 (-7.24, 5.83)	0.833	-1.80 (-9.69, 6.09)	0.654	2.29 (-1.16, 5.74)	0.193	-4.47 (-19.60, 10.66)	0.563
	Overweight	-7.74 (-22.80, 7.31)	0.313	-6.31 (-17.88, 5.25)	0.285	-7.08 (-21.00, 6.83)	0.318	-1.39 (-7.48, 4.71)	0.656	6.86 (-20.44, 34.16)	0.622
	Obese	-20.04 (-68.24, 28.15)	0.415	-8.47 (-45.18, 28.24)	-0.651	-13.82 (-58.21, 30.56)	0.542	-5.97 (-25.44, 13.50)	0.548	-29.22 (-114.01, 55.57)	0.499
Duration of ART use at recruitment	**<2.5 years**	Comparison		Comparison		Comparison		Comparison		Comparison	
	2.5 - 4.9 years	-4.63 (-15.35, 6.10)	0.398	-4.15 (-12.36, 4.06)	0.322	-10.82 (-20.91, -0.73)	0.036	5.36 (0.75, 9.96)	0.023	-25.35 (-46.16, -4.54)	0.017
	5.0 – 7.4 years	2.15 (-9.03, 13.33)	0.706	-0.19 (-8.82, 8.43)	0.965	-4.24 (-14.77, 6.29)	0.430	6.79 (1.98, 11.60)	0.006	-16.74 (-38.52, 5.04)	0.132
	7.5 – 9.9 years	-4.76 (-15.77, 6.25)	0.397	-6.46 (-14.92, 1.99)	0.134	-8.62 (-19.00, 1.75)	0.103	0.64 (-4.09, 5.38)	0.789	8.10 (-13.21, 29.41)	0.456
	≥10 years	-24.35 (-36.17, -12.53)	<0.001	-22.20 (-31.22, -13.18)	<0.001	-26.60 (-37.66, -15.54)	<0.001	1.55 (-3.50, 6.60)	0.548	-12.43 (-35.12, 10.26)	0.283
Recruitment protease inhibitor	**No use**	Comparison		Comparison		Comparison		Comparison		Comparison	
	Use	14.93 (7.66, 22.20)	<0.001	12.35 (6.73, 17.98)	<0.001	21.77 (15.16, 28.39)	<0.001	-5.72 (-8.78, -2.67)	<0.001	49.05 (36.36, 61.75)	<0.001
Any protease inhibitor use over follow-up	**Never use**	Comparison		Comparison		Comparison		Comparison		Comparison	
	Ever use	14.51 (7.20, 21.81)	<0.001	13.30 (7.67, 18.93)	<0.001	23.36 (16.77, 29.96)	<0.001	-6.50 (-9.56, -3.45)	<0.001	51.86 (39.22, 64.49)	<0.001
Recruitment nucleoside reverse transcriptase inhibitor	**No use**	Comparison		Comparison		Comparison		Comparison		Comparison	
	Use	13.76 (-0.33, 27.84)	0.056	8.30 (-2.73, 19.33)	0.140	8.96 (-4.37, 22.28)	0.188	4.42 (-1.49, 10.33)	0.143	11.75 (-14.04, 37.54)	0.372
Any nucleoside reverse transcriptase inhibitor use over follow-up	**Never use**	Comparison		Comparison		Comparison		Comparison		Comparison	
	Ever use	-10.80 (-89.71, 68.11)	0.789	-6.51 (-65.52, 52.50)	0.829	-29.69 (-100.43, 41.06)	0.411	18.77 (-12.65, 50.18)	0.242	-115.84 (-255.22, 23.54)	0.103

		Total cholesterol		LDL-cholesterol		Non-HDL cholesterol		HDL cholesterol		Fasting triglyceride	
		Beta (95% CI)	*p*-value	Beta (95% CI)	*p*-value	Beta (95% CI)	*p*-value	Beta (95% CI)	*p*-value	Beta (95% CI)	*p*-value
Recruitment reverse non-nucleoside reverse transcriptase inhibitor	**No use**	Comparison		Comparison		Comparison		Comparison		Comparison	
	Use	-3.53 (-11.46, 4.40)	0.383	-5.57 (-11.71, 0.57)	0.075	-13.06 (-20.45, -5.67)	0.001	7.91 (4.66, 11.15)	<0.001	-35.70 (-50.24,-21.17)	<0.001
Any non-nucleoside reverse transcriptase inhibitor use over follow-up	**Never use**	Comparison		Comparison		Comparison		Comparison		Comparison	
	Ever use	21.79 (10.34, 33.24)	<0.001	11.27 (2.29, 20.25)	0.014	17.62 (6.72, 28.52)	0.002	3.24 (-1.65, 8.13)	0.194	30.80 (9.83, 51.77)	0.004
ART regimen at recruitment	**PI-based HAART**	Comparison		Comparison		Comparison		Comparison		Comparison	
	NRTI mono-therapy	-22.01 (-36.08, -7.94)	0.002	-16.77 (-27.48, -6.05)	0.002	-26.98 (-39.84, -14.13)	<0.001	4.87 (-1.01, 10.75)	0.104	-51.78 (-76.62, -26.95)	<0.001
	NNRTI-based HAART	-10.07 (-18.35, -1.79)	0.017	-10.05 (-16.51, -3.59)	0.002	-20.25 (-27.85, -12.65)	<0.001	8.34 (4.90, 11.79)	<0.001	-49.84 (-64.66, -35.02)	<0.001
	Triple class therapy	9.42 (-9.37, 28.22)	0.326	0.76 (-13.65, 15.16)	0.918	-0.82 (-17.67, 16.02)	0.924	7.03 (-0.60, 14.67)	0.071	4.16 (-28.44, 36.77)	0.802
Time-updated ART regiment	**PI-based HAART**	Comparison		Comparison		Comparison		Comparison		Comparison	
	NRTI mono-therapy	-16.59 (-25.78, -7.40)	<0.001	-12.90 (-19.91, -5.89)	<0.001	-19.08 (-27.45, -10.70)	<0.001	1.04 (-2.71, 4.80)	0.585	-40.21 (-57.43, -23.00)	<0.001
	NNRTI-based HAART	-9.75 (-17.29, -2.20)	0.001	-10.48 (-16.40, -4.56)	0.001	-20.61 (-27.59, -13.64)	<0.001	8.08 (4.92, 11.23)	<0.001	-48.18 (-61.71, -34.65)	<0.001
	Triple class therapy	1.66 (-6.61, .93)	0.694	-2.81 (-8.87, 3.24)	0.362	-4.25 (-11.58, 3.08)	0.256	2.97 (-0.26, 6.20)	0.071	2.45 (-15.03, 19.93)	0.784

3-level models include repeated measures of individuals who are clustered into hospital in the random intercept

G.2 Multivariable multilevel models for cholesterol and fasting triglyceride

Table G-3: Multivariable multilevel model for cholesterol and fasting triglyceride including recruitment explanatory variables

		Total cholesterol *n* = 212 observations = 602		LDL-cholesterol *n* = 209 observations = 559		Non-HDL cholesterol *n* = 208 observations = 560		HDL cholesterol *n* = 208 observations = 561		Fasting triglyceride *n* = 210 observations = 522	
		Estimate	***p – value***	**Estimate**	***p - value***	**Estimate**	***p - value***	**Estimate**	***p - value***	**Estimate**	***p - value***
Age at recruitment	**<12 years**	Comparison		Comparison		Comparison		Comparison		Comparison	
	12-18 years	-15.0 (-25.5, -4.4)	0.006	-7.2 (-15.4, 1.0)	0.086	-9.7 (-19.2, -0.2)	0.045	-2.9 (-7.0, 1.2)	0.170	-4.2 (-22.8, 14.5)	0.663
Sex	**Male**	Comparison		Comparison		Comparison		Comparison		Comparison	
	Female	2.4 (-6.8, 11.6)	0.613	3.8 (-3.4, 10.9)	0.301	-1.9 (-10.1, 13.3)	0.655	5.3 (1.7, 8.8)	0.004	-23.9 (-40.3, -7.5)	0.004
Ethnicity	**Black**	Comparison		Comparison		Comparison		Comparison		Comparison	
	White	-13.9 (-25.6, -2.2	0.019	-7.1 (-16.0, 1.7)	0.112	-5.1 (-15.3, 5.0)	0.320	-7.3 (-12.1, -2.6)	0.003	18.1 (-1.8, 38.0)	0.075
	Other	-20.5 (-44.1, 3.1)	0.089	-11.1 (-29.3, 7.1)	0.232	-7.6 (-28.6, 13.3)	0.475	-10.2 (-19.4, -0.9)	0.031	12.9 (-28.7, 54.4)	0.544
Body mass index at recruitment	**Healthy weight**	Comparison		Comparison		Comparison		Comparison		Comparison	
	Severely underweight	5.4 (-8.9, 19.7)	0.462	10.5 (-0.5, 21.6)	0.062	2.2 (-10.5, 14.9)	0.734	3.4 (-2.2, 9.0)	0.238	-27.0 (-52.4, -1.6)	0.038
	Underweight	-0.5 (-11.5, 10.6)	0.934	-2.4 (-11.0, 6.2)	0.587	-2.7 (-12.7, 7.2)	0.589	0.6 (-3.7, 5.0)	0.773	0.7 (-19.0, 20.4)	0.942
	Overweight	1.2 (-17.3, 19.6)	0.899	-1.4 (-15.7, 12.9)	0.847	-3.5 (-20.0, 13.0)	0.677	2.4 (-4.7, 9.6)	0.503	-3.0 (-35.9, 29.8)	0.856
	Obese	-35.7 (-99.6, 28.2)	0.273	-26.7 (-75.6, 22.3)	0.286	-43.6 (-100.2, 13.0)	0.131	3.7 (-21.0, 28.3)	0.769	-89.7 (-199.9, 20.5)	0.111
Lipodystrophy syndrome at recruitment	**Absent**	Comparison		Comparison		Comparison		Comparison		Comparison	
	Present	20.5 (10.0, 31.0)	<0.001	11.1 (2.9, 19.2)	0.008	19.7 (10.3, 29.1)	<0.001	0.8 (-3.3, 4.9)	0.710	37.0 (18.5, 55.5)	<0.001
Maximum clinical condition	**N/A**	Comparison		Comparison		Comparison		Comparison		Comparison	
	B	11.6 (0.8, 22.4)	0.036	9.6 (1.2, 18.0)	0.024	11.0 (1.3, 20.8)	0.026	1.1 (-3.1, 5.3)	0.617	11.0 (-8.2, 30.2)	0.262
	C	6.0 (-6.3, 18.3)	0.340	6.4 (-3.1, 16.0)	0.189	5.6 (-5.4, 16.6)	0.318	0.3 (-4.5, 5.1)	0.890	-6.2 (-28.3, 15.9)	0.581
Immuno-suppression at recruitment	**None**	Comparison		Comparison		Comparison		Comparison		Comparison	
	Moderate	-8.9 (-21.3, 3.6)	0.162	-4.1 (-13.7, 5.5)	0.402	-4.3 (-15.4, 6.8)	0.451	-4.1 (-8.9, 0.8)	0.101	-6.2 (-28.2, 15.9)	0.584
	Severe	-9.2 (-42.6, 24.1)	0.587	-12.3 (-38.1, 13.5)	0.250	-7.8 (-37.6, 22.0)	0.609	-4.5 (-17.5, 8.5)	0.500	35.1 (-23.4, 93.6)	0.240

		Total cholesterol *n* = 212 observations = 602		LDL-cholesterol *n* = 209 observations = 559		Non-HDL cholesterol *n* = 208 observations = 560		HDL cholesterol *n* = 208 observations = 561		Fasting triglyceride *n* = 210 observations = 522	
		Estimate	***p – value***	**Estimate**	***p - value***	**Estimate**	***p - value***	**Estimate**	***p - value***	**Estimate**	***p - value***
Detectable viral load	**<50 copies/ml**	Comparison		Comparison		Comparison		Comparison		Comparison	
	≥50 copies/ml	-13.9 (-27.5, -0.3)	0.045	-3.4 (-13.9, 7.0)	0.518	-7.2 (-19.3, 5.0)	0.247	-6.1 (-11.8, -0.5)	0.034	-11.2 (-34.9, 12.4)	0.352
ART regimen at recruitment	**PI-based HAART**	Comparison		Comparison		Comparison		Comparison		Comparison	
	NRTI mono - therapy	-13.9 (-30.7, 2.9)	0.105	-12.5 (-25.3, 0.4)	0.057	-17.6 (-32.4, -2.9)	0.019	4.7 (-1.9, 11.2)	0.164	-37.4 (-67.1, -7.8)	0.014
	NNRTI-based HAART	-9.2 (-19.7, 1.3)	0.087	-6.6 (-14.8, 1.6)	0.116	-16.2 (-25.7, -6.6)	<0.001	7.3 (3.1, 11.4)	0.001	-45.4 (-64.0, -26.8)	<0.001
	Triple-class therapy	3.1 (-17.2, 23.4)	0.764	-4.7 (-20.3, 10.9)	0.558	-6.6 (-24.6, 11.5)	0.476	6.6 (-1.3, 14.5)	0.100	-3.2 (-39.0, 32.5)	0.860
Duration of ART use at recruitment	**<2.5 years**	Comparison		Comparison		Comparison		Comparison		Comparison	
	2.5 - 4.9 years	-5.1 (-18.7, 8.5)	0.461	-4.2 (-14.7, 6.2)	0.426	-11.3 (-23.4, 0.8)	0.066	5.6 (0.2, 10.9)	0.041	-22.3 (-46.4, 1.7)	0.069
	5.0 – 7.4 years	-5.5 (-20.0, 8.9)	0.453	-1.5 (-12.7, 9.7)	0.795	-8.5 (-21.5, 4.5)	0.200	4.3 (-1.4, 9.9)	0.139	-18.0 (-43.7, 7.6)	0.168
	7.5 – 9.9 years	-6.9 (-20.6, 6.9)	0.328	-4.3 (-15.0, 6.3)	0.426	-8.3 (-20.7, 4.0)	0.187	-0.8 (-6.2, 4.6)	0.784	2.4 (-22.2, 26.9)	0.849
	≥10 years	-10.3 (-26.3, 5.6)	0.205	-12.8 (-25.2, -0.5)	0.041	-16.2 (-30.5, -2.0)	0.025	5.5 (-0.8, 11.8)	0.085	-5.5 (-33.7, 22.7)	0.702
Intercept		195.1 (173.3, 217.1)	<0.001	105.8 (89.2, 122.5)	<0.001	137.1 (118.0, 156.2)	<0.001	55.5 (46.7, 64.4)	<0.001	127.5 (89.8, 165.3)	<0.001

Random effect statistics provided in Table

Table G-4: Estimates of random effects' standard deviations for multivariable multilevel models with recruitment explanatory variables

	Clinical site		Individual		Residual	
	Est.	**95% CI**	**Est.**	**95% CI**	**Est.**	**95% CI**
Total cholesterol	3.62	(0.40, 32.41)	28.68	(25.36, 32.43)	23.20	(21.63, 24.88)
LDL cholesterol	1.30	(0, 54286.70)	21.88	(19.20, 24.92)	18.21	(16.92, 19.60)
Non-HDL cholesterol	0.00	(0.00, 41.93)	25.66	(22.68, 29.03)	19.92	(18.43, 21.53)
HDL cholesterol	2.50	(0.68, 9.24)	11.03	(9.73, 12.52)	8.70	(8.08, 9.36)
Fasting triglyceride	0.00	(0.00, 0.17)	46.18	(36.95, 57.71)	50.86	(46.79, 55.29)

Table G-5: Multivariable multilevel model for cholesterol and fasting triglyceride including time-updated explanatory variables

		Total cholesterol *n* = 253 observations = 668		LDL-cholesterol *n* = 250 observations = 619		Non-HDL cholesterol *n* = 249 observations = 621		HDL cholesterol *n* = 249 observations = 622		Fasting triglyceride *n* = 250 observations = 574	
		Estimate	*p – value*	Estimate	*p - value*	Estimate	*p - value*	Estimate	*p - value*	Estimate	*p - value*
Age at recruitment	**<12 years**	Comparison		Comparison		Comparison		Comparison		Comparison	
	12-18 years	-12.4 (-21.5, -3.3)	0.007	-5.7 (-12.8, 1.4)	0.118	-7.5 (-15.6, 0.7)	0.073	-2.9 (-6.6, 0.8)	0.123	-4.4 (-21.8, 13.0)	0.621
Sex	**Male**	Comparison		Comparison		Comparison		Comparison		Comparison	
	Female	3.0 (-4.9, 11.0)	0.455	2.9 (-3.3, 9.1)	0.357	-1.9 (-9.0, 5.2)	0.601	5.2 (1.9, 8.4)	0.002	-20.0 (-34.8, -4.5)	0.011
Ethnicity	**Black**	Comparison		Comparison		Comparison		Comparison		Comparison	
	White	-6.9 (-16.7, 2.9)	0.168	-3.5 (-11.2, 4.2)	0.371	0.7 (-8.1, 9.6)	0.868	-7.3 (-11.3, -3.3)	<0.001	27.4 (8.9, 45.9)	0.004
	Other	-19.1 (-39.3, 1.2)	0.065	12.6 (-28.4, 3.1)	0.116	-9.0 (-26.9, 9.0)	0.326	-8.4 (-16.6, -0.3)	0.043	14.3 (-23.4, 51.9)	0.458
Body mass index over follow-up	**Healthy weight**	Comparison		Comparison		Comparison		Comparison		Comparison	
	Severely underweight	10.9 (1.5, 20.4)	0.023	8.8 (1.2, 16.4)	0.023	6.7 (-1.7, 15.1)	0.118	4.1 (0.3, 7.9)	0.035	-17.1 (-37.5, 3.3)	0.101
	Underweight	-0.6 (-7.3, 6.1)	0.863	-2.0 (-7.5, 3.5)	0.468	-4.3 (-10.3, 1.7)	0.162	1.8 (-0.9, 4.5)	0.189	3.5 (-11.5, 18.4)	0.650
	Overweight	7.6 (-3.1, 18.2)	0.163	1.0 (-7.7, 9.6)	0.829	5.2 (-4.4, 14.8)	0.287	-0.4 (-4.8, 3.9)	0.842	8.8 (-13.8, 31.5)	0.445
	Obese	-33.5 (-105.4, 38.3)	0.361	-25.1 (-80.7, 30.5)	0.376	-49.0 (-111.7, 13.7)	0.126	12.0 (-16.5, 40.5)	0.410	-114.2 (-252.7, 24.3)	0.106
Lipodystrophy syndrome over follow-up	**Absent**	Comparison		Comparison		Comparison		Comparison		Comparison	
	Present	19.4 (8.2, 30.6)	0.001	12.6 (3.8, 21.3)	0.005	19.3 (9.3, 29.4)	<0.001	0.5 (-4.1, 5.1)	0.824	37.3 (16.3, 58.2)	<0.001
Maximum clinical condition	**N/A**	Comparison		Comparison		Comparison		Comparison		Comparison	
	B	6.7 (-2.8, 16.2)	0.169	4.6 (-2.8, 12.0)	0.224	5.8 (-2.8, 14.3)	0.185	0.55 (-3.3, 4.4)	0.782	8.6 (-9.3, 26.4)	0.348
	C	4.7 (-5.7, 15.2)	0.376	3.9 (-4.3, 12.1)	0.349	3.2 (-6.2, 12.5)	0.505	0.5 (-3.7, 4.8)	0.814	-7.6 (-27.8, 12.5)	0.457
Immuno-suppression over follow-up	**None**	Comparison		Comparison		Comparison		Comparison		Comparison	
	Any	-8.9 (-17.3, -0.4)	0.400	-4.2 (-10.8, 2.4)	0.211	-6.9 (-14.4, 0.7)	0.075	-2.3 (-5.7, 1.2)	0.198	-9.5 (-25.5, 6.5)	0.246
Detectable viral load over follow-up	**None**	Comparison		Comparison		Comparison		Comparison		Comparison	
	Any	-15.6 (-27.5, -3.7)	0.010	-6.4 (-15.8, 2.9)	0.178	-10.6 (-21.4, 0.2)	0.055	-5.5 (-10.4, -0.5)	0.030	-9.9 (-32.7, 12.9)	0.396
ART regimen over follow-up	**PI-based HAART**	Comparison		Comparison		Comparison		Comparison		Comparison	
	NRTI mono - therapy	-13.4 (-24.4, -2.4)	0.017	-12.5 (-21.0, -3.9)	0.023	-17.3 (-27.0, -7.7)	<0.001	3.0 (-1.3, 7.4)	0.172	-33.5 (-55.5, -11.6)	0.003
	NNRTI-based HAART	-4.7 (-13.5, 4.2)	0.163	-5.9 (-13.0, 1.2)	0.104	-14.7 (-22.7, -6.6)	<0.001	8.4 (4.7, 12.1)	<0.001	-47.1 (-64.4, -29.8)	<0.001

		Total cholesterol *n* = 253 observations = 668		LDL-cholesterol *n* = 250 observations = 619		Non-HDL cholesterol *n* = 249 observations = 621		HDL cholesterol *n* = 249 observations = 622		Fasting triglyceride *n* = 250 observations = 574	
		Estimate	***p – value***	**Estimate**	***p - value***	**Estimate**	***p - value***	**Estimate**	***p - value***	**Estimate**	***p - value***
	Triple-class therapy	1.7 (-7.1, 10.6)	0.702	-3.5 (-10.5, 3.6)	0.332	-4.8 (-12.5, 2.9)	0.223	4.2 (0.7, 7.7)	0.018	0.3 (-20.8, 21.4)	0.980
Duration of ART use at recruitment	**<2.5 years**	Comparison		Comparison		Comparison		Comparison		Comparison	
	2.5 - 4.9 years	-7.2 (-19.0, 4.5)	0.229	-6.0 (-15.2, 3.2)	0.199	-13.9 (-24.4, -3.3)	0.010	5.7 (0.9, 10.4)	0.021	-26.4 (-48.9, -4.0)	0.021
	5.0 – 7.4 years	-4.0 (-16.6, 8.6)	0.535	-2.9 (-12.7, 7.0)	0.571	-10.1 (-21.4, 1.2)	0.079	6.8 (1.6, 11.9)	0.010	-18.9 (-42.6, 4.8)	0.118
	7.5 – 9.9 years	-9.1 (-20.8, 2.6)	0.129	-6.8 (-16.0, 2.4)	0.149	-11.3 (-21.9, -0.8)	0.035	0.2 (-4.6, 5.0)	0.824	-0.7 (-23.0, 21.6)	0.950
	≥10 years	-9.1 (-22.7, 4.5)	0.190	-12.1 (-22.8, -1.5)	0.026	-15.5 (-27.7, -3.3)	0.013	6.0 (0.4, 11.5)	0.035	-7.8 (-33.6, 18.0)	0.552
Intercept		189.4 (169.6, 209.1)	<0.001	106.6 (91.1, 122.2)	0.005	136.2 (118.4, 154.0)	<0.001	54.4 (46.4, 62.5)	<0.001	115.9 (78.4, 153.4)	<0.001

Random effect statistics provided in Table G-6

Table G-6: Estimates of random effects' standard deviations for multivariable multilevel models with time-updated explanatory variables

	Clinical site		Individual		Residual	
	Est.	**95% CI**	**Est.**	**95% CI**	**Est.**	**95% CI**
Total cholesterol	0.00	(0.00, 0.00)	27.34	(24.36, 30.69)	23.60	(22.06, 25.25)
LDL cholesterol	0.00	(0.00, 9.05)	20.96	(18.25, 24.08)	18.43	(16.73, 20.30)
Non-HDL cholesterol	0.00	(0.00, 18.59)	24.47	(21.76, 27.52)	19.84	(18.40, 21.39)
HDL cholesterol	0.00	(0.00, -)	11.17	(9.95, 12.53)	8.93	(8.31, 9.60)
Fasting triglyceride	0.00	(0.00, 0.00)	47.01	(40.51, 54.55)	51.37	(47.52, 55.53)

3-level model includes individuals clustered into clinical site in the random intercept. 2-level model includes subjects in the random intercept

Table G-7: Comparison of 3-level and 2-level models with log ratio tests

	Models adjusted by categories of ART				Models adjusted by ART regimen			
	Recruitment explanatory factors		Time-updated explanatory factors		Recruitment explanatory factors		Time-updated explanatory factors	
	χ^2	*p*-value	χ^2	*p*-value	χ^2	*p*-value	χ^2	*p*-value
Total cholesterol	0.598	0.493	26.480	<0.001	0.259	0.611	17.644	<0.001
LDL cholesterol	0.000	1.000	24.920	<0.001	0.006	0.938	16.837	<0.001
Non-HDL cholesterol	0.000	1.000	26.637	<0.001	0.000	1.000	0.001	1.000
HDL cholesterol	0.144	0.704	21.630	<0.001	1.024	0.311	14.853	<0.001
Fasting triglyceride	0.000	1.000	31.196	<0.001	0.000	1.000	20.781	<0.001

Table G-8: Comparison of 3-level and null models with log ratio tests

	Models adjusted by categories of ART				Models adjusted by ART regimen			
	Recruitment explanatory factors		Time-updated explanatory factors		Recruitment explanatory factors		Time-updated explanatory factors	
	χ^2	*p*-value	χ^2	*p*-value	χ^2	*p*-value	χ^2	*p*-value
Total cholesterol	155.847	<0.001	241.941	<0.001	103.624	<0.001	117.505	<0.001
LDL cholesterol	164.877	<0.001	231.483	<0.001	87.972	<0.001	108.069	<0.001
Non-HDL cholesterol	199.742	<0.001	258.453	<0.001	108.103	<0.001	133.956	<0.001
HDL cholesterol	213.074	<0.001	213.525	<0.001	128.832	<0.001	151.586	<0.001
Fasting triglyceride	10.664	<0.005	31.196	<0.001	67.252	<0.001	32.208	<0.001

3-level model includes individuals clustered into clinical site in the random intercept. null model includes no random intercept

Appendix H Appendix to Chapter 8

H.1 Observational adult studies investigating lipodystrophy syndrome

Table H-1: Methods of lipodystrophy assessment in observational adult studies cited in this thesis

Study	Study type	Location	Number of subjects	Age	Assessment of outcomes
Andany *et al* (2011) *Ontario Cohort Study*	Multi-centre cross-sectional	Canada	778	48 (median)	Self-assessed lipoatrophy and/or lipohypertrophy
Bonfanti *et al* (2003)[404] *Coordinamento Italiano Studio Allergia e Infezione da HIV (CISAI) Group*	Multi-centre, 22-month prospective	Italy	1480	Adults (not specified)	Clinician-assessed lipoatrophy and/or lipohypertrophy
Bongiovanni *et al* (2006)[402]	3-month prospective	Italy	382	38 (median)	Non-HDL cholesterol ≥190mg/dL Triglyceride ≥200mg/dL
Bogner *et al* (2001)[403]	101-week prospective	Germany	115	38 (mean)	Lipoatrophy and/or lipohypertrophy by >2 of: assessed by patient, assessed by clinician, or by treatment-blinded physician
Calza *et al* (2003)[410]	1-year prospective	Italy	212	38 (mean)	Cholesterol ≥200mg/dL (severe ≥300mg/dL) HDL-cholesterol ≤35mg/dL LDL-cholesterol ≥160mg/dL Triglyceride ≥200mg/dL (severe ≥750 mg/dL)
Carr *et al* (1999)[226]	<27 week prospective	United States	113	40 (mean)	Self-assessed body fat alterations Cholesterol >213 mg/dL HDL-cholesterol <35mg/dL Triglyceride >177 mg/dL
Carter *et al* (2001)[411]	Cross-sectional	Australia	159 males	Not specified	Self-reported lipoatrophy and/or lipohypertrophy Body fat alterations: total body fat by DEXA<20%, or waist circumference >90cm Fasting cholesterol >213mg/dL Fasting triglyceride >177 mg/dL

Study	Study type	Location	Number of subjects	Age	Assessment of outcomes
Dong *et al* (1999)[583]	Cross-sectional	United States	21 females	39 (mean)	Abdominal lipohypertrophy: increased belt/dressed size Breast lipohypertrophy: increased bra size Peripheral lipoatrophy: visual definition of muscle group and increased prominence of veins
Friis-Moller *et al* (2004)[394] Data collection on Adverse events of anti-HIV Drugs (DAD) study	Multi-centre cross-sectional	Multi-country	17,852	39 (median)	Clinician-assessed body fat alterations Dyslipidemia: total cholesterol ≥240mg/dL and/or HDL-cholesterol ≤35mg/dL and/or triglycerides ≥200mg/dL
FRAM (2005)[584] *The Study of Fat Redistribution and Metabolic Change in HIV Infection*	Multi-centre cross-sectional	United States	425 males	40 (median)	Self-assessed and researcher-validated lipoatrophy and/or lipohypertrophy, magnetic resonance imagining to measure compartmental percentage fat
FRAM (2006)[434] *The Study of Fat Redistribution and Metabolic Change in HIV Infection*	Multi-centre cross-sectional	United States	183 females	39 (median)	Self-assessed and researcher-validated lipoatrophy and/or lipohypertrophy, magnetic resonance imagining to measure compartmental percentage fat
Galli *et al* (2002)[407]	25-week prospective study	Italy	335	34 (median)	Self and clinician assessed lipoatrophy and lipohypertrophy : subset validated by DEXA Cholesterol ≥250mg/dL Triglyceride ≥200mg/dL
Galli *et al* (2002)[421] LipoICoNa (Lipo-Italian Cohort of Antiretroviral-Naïve patients)	Multi-centre 86-week prospective	Italy	655	35 (median)	Physician-assessed lipoatrophy and/or lipohypertrophy
George *et al* (2009)[224]	2-year prospective	South Africa	42	34 (mean)	Self and clinician-assessed lipoatrophy and lipohypertrophy Cholesterol ≥193mg/dL LDL-cholesterol ≥116mg/dL HDL-cholesterol <39mg/dL Triglyceride ≥151mg/dL
Gervasoni *et al* (1999)[228]	Cross-sectional	Italy	306	34 (median)	Clinical observations to observe changes in body habitus from previous hospital appointment.

Study	Study type	Location	Number of subjects	Age	Assessment of outcomes
					Patients with body fat alterations underwent DEXA to measure percentages body fat and lean body mass
Heath *et al* (2002)[406]	1-year prospective	Canada	745	41 (mean)	Self-assessed lipoatrophy and/or lipohypertrophy Total cholesterol >201 mg/dL Fasting triglyceride 204mg/dL
Heath *et al* (2002)[422]	1-year prospective	Canada	366	Adult (not specified)	Self-assessed lipoatrophy and/or lipohypertrophy Total cholesterol >201 mg/dL Fasting triglyceride >204mg/dL
Jacobson *et al* (2005)[413] *Nutrition for Healthy Living longitudinal study of HIV-infected adults*	1-year prospective	United States	452	44 (mean men) 41 (mean women)	Lipoatrophy by triceps skin fold measurement < 10% NHANTES by sex and gender Lipohypertrophy by waist/hip ratio (>0.95 men, >0.85 women)
Jacobson *et al* (2006)[408] *Nutrition for Healthy Living longitudinal study of HIV-infected adults*	Cross-sectional	United States	477	43 (median)	Abdominal obesity: waist circumference >102cm (men)/88cm (women) HDL-cholesterol <40mg/dL Triglyceride >150mg/dL
Jevtovic et al (2009)[412]	Cross-sectional	Serbia	582	45 (mean)	Self and clinician-assessed lipoatrophy and/or lipohypertrophy Cholesterol ≥240 mg/dL HDL-cholesterol ≤166 mg/dL L LDL-cholesterol ≥159 mg/dL Triglycerides ≥159 mg/dL
Lichtenstein *et al* (2001)[215] *HIV Outpatient Study (HOPS)*	Multi-centre cross-sectional	United States	1077	41 (median)	Clinician assessed lipoatrophy and lipohypertrophy
Miller *et al* (2003)[405]	Multi-centre cross-sectional	Australia	1348	39.8 (mean)	Clinician-assessed lipoatrophy and/or lipohypertrophy : subset examined by DEXA and CAT scans
Martinez *et al* (2001)[199]	18-month prospective	Spain	494	Adults (not specified)	Clinician-assessed lipoatrophy and/or lipohypertrophy
Mallal *et al* (2000)[235] *Western Australian Study HIV Cohort*	30-month prospective	Australia	277	41 (mean)	Patient and clinical assessment of lipoatrophy Percentage of fat assessed by DEXA scans
Mauss *et al* (2002)	36-month multi-	Germany	221	38 (median)	Clinician-assessed lipoatrophy and/or lipohypertrophy with use of

Study	Study type	Location	Number of subjects	Age	Assessment of outcomes
DAGNAE LipART	centre prospective				visual analogue scales
Mutimura *et al* (2007)[193]	Cross-sectional	Rwanda	571	38 (mean)	Body fat alterations confirmed by physician Cholesterol >240mg/dL HDL-cholesterol <39mg/dL Triglyceride >195mg/dL
Narciso *et al* (2001)[420]	1-year prospective	Italy	41	31 (mean)	Self-assessed body fat alterations Cholesterol >200mg/dL Triglyceride >220
Paton *et al* (2002)[401]	Cross-sectional	Singapore	410	40 (mean)	Self-assessed body fat alterations
Pujari *et al* (2005)[227]	Cross-sectional	India	306	37 (mean)	Self-assessed lipoatrophy and lipohypertrophy confirmed by physician Cholesterol ≥200 mg/dL LDL-cholesterol ≥130mg/dL Triglycerides ≥150mg/dL HDL-cholesterol <40mg/dL Total cholesterol: HDL-cholesterol ratio ≥6.5
Tien *et al* (2003)[423]	30-month prospective	United States	605 women	41 (median)	Examiner-assessed lipoatrophy and/or lipohypertrophy (self-assessed changes confirmed by anthropometric measures)
Saves *et al* (2002)[225] *Antiproteases Cohorte (APROCO)*	Multi-centre cross-sectional	France	614	Adults (not specified)	Clinician-assessed lipoatrophy and/or lipohypertrophy Cholesterol ≥193mg/dL HDL-cholesterol <35mg/dL LDL-cholesterol ≥131mg/dL Triglyceride ≥195mg/dL
Saint-Marc *et al* (2000)[184] *LIPOCO Study*	Multi-centre cross-sectional	France	154 male	40 (mean)	Regional fat distribution measured by computed tomography (CT): intra-abdominal visceral and subcutaneous adipose
Seminari *et al* (2002)[463]	Cross-sectional	Italy	504	38 (mean)	Clinician-assessed lipoatrophy and/or lipohypertrophy Graded cholesterol: grade 1 (200-239mg/dL), grade 2 (240-300mg/dL), and grade 3 (≥300mg/dL) Graded triglyceride: grade 1 (200-399mg/dL), grade 2 (400-1000mg/dL), and grade 3 (>1000mg/dL)
Shlay *et al* (2009)[236] *The Metabolic Study*	Cross-sectional	United States	416	38 (mean)	Measures of subcutaneous and non-subcutaneous tissue estimated from calculation of recorded circumferences (arm, thigh, waist) and skinfold thickness (subscapular, suprascapular, thigh, triceps and abdomen)

Study	Study type	Location	Number of subjects	Age	Assessment of outcomes
Thiebaut *et al* (2000)[216]	Multi-centre cross-sectional	France	581	39 (med)	Lipoatrophy and/or lipohypertrophy assessed by physician Cholesterol > 213mg/dL Fasting triglyceride >531mg/dL
Thiebaut *et al* (2001)[409] *French Aquitaine Cohort*	Multi-centre 25-month prospective	France	925	36 (median)	Triglyceride >531mm/L Hypercholesterolemia (threshold not specified)
Tsiodras *et al* (2000)[398] *French Aquitaine Cohort*	5-year retrospective cohort	United States	221	37	Cholesterol >240mg/dL Triglyceride > 500mg/dL
Van Griensven *et al* (2007)[399]	Cross-sectional	Rwanda	409	38 (median)	Self-reported and clinical assessment of lipoatrophy
Walmsley *et al* (2008)[499]	36-month prospective	Canada	68	39 (median)	Self and clinician-assessed lipoatrophy and/or lipohypertrophy (with photography, regional DEXA scan)
Young *et al* (2005)[447] *Swiss HIV Cohort Study*	20-month prospective	Switzerland	925	Adults (not specified)	Lipoatrophy and/or lipohypertrophy assessment by clinician and patient
Zannou *et al* (2009)[400]	23.2 month prospective	Benin	88	38 (mean)	Physician-assessed lipoatrophy and/or lipoatrophy Metabolic syndrome (thresholds of waist circumference,/elevated triglyceride/elevated blood pressure/elevated glucose/reduced HDL-cholesterol

Lipoatrophy and/or lipohypertrophy denotes fat alterations in specific body locations, however these locations may not be consistent between all studies